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Potential Orally-Active Heparin-Like Compounds: Synthesis and Anticoagulant Activity

Dissertação do 2º Ciclo de Estudos Conducente ao Grau de Mestre em Química Farmacêutica

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Author's declaration:

Under the terms of the Decree-Law nº 216/92, of October 13th, is hereby declared that the author afforded a major contribution to the conceptual design and technical execution of the work and interpretation of the results included in this dissertation. Under the terms of the referred Decree-Law, is hereby declared that the following articles/communications were prepared in the scope of this dissertation.

The results presented in this dissertation are part of the following scientific communications:

Poster Communications in Conferences

Internationals:

1. **Neves, A. R.***; Correia-da-Silva, M.; Sousa, E.; Pinto, M. Heparin-like small-molecules: reaching oral bioavailability. EFMC Young Medicinal Chemist Symposium, Antwerp, Belgium, September 17, 2015 (accepted).

2. **Neves, A. R.***; Correia-da-Silva, M.; Sousa, E.; Pinto, M. Development of conjugates to improve oral bioavailability of sulfated small molecules. P QS49. XX *Encontro Luso-Galego de Química*, Porto, Portugal, November 26-28, 2014.

Nationals:

3. **Neves, A.R.***; Correia-da-Silva, M.; Sousa, E.; Duarte, P.; Silva, P; Bousbaa, H.; Pinto, M. Acetylated flavonoids and xanthones: synthesis and screening for antitumor activity. III *Encontro Nacional de Estudantes de Química*, Aveiro, Portugal, March 27-29, 2015.

4. **Neves, A. R.***; Correia-da-Silva, M.; Sousa, E.; Pinto, M. Synthesis of a sulfated flavonoid - deoxycholic acid conjugate as a potential orally-active antithrombotic agent. 8º *Encontro de Jovens Investigadores da Universidade do Porto* (IJUP15), Porto, Portugal, May 13-15, 2015.

5. Zovinec, M.*; **Neves, A. R.**; Correia-da-Silva, M.; Sousa, E.; Pinto, M. Carboxymethylation of flavonoids: synthesis, purification and structure elucidation. 8º *Encontro de Jovens Investigadores da Universidade do Porto* (IJUP15), Porto, Portugal, May 13-15, 2015.

Abstracts in Conferences Proceedings

Internationals:

1. **Neves, A. R.***; Correia-da-Silva, M.; Sousa, E.; Pinto, M. Development of conjugates to improve oral bioavailability of sulfated small molecules. XX *Encontro Luso-Galego de Química no Porto*, 2014, p. 382.

Nationals:

2. **Neves, A. R.***; Correia-da-Silva, M.; Sousa, E.; Pinto, M. Synthesis of a sulfated flavonoid - deoxycholic acid conjugate as a potential orally-active antithrombotic agent. *8º Encontro de Jovens Investigadores da Universidade do Porto* (IJUP15), 2015, p. 277.

3. Zovinec, M.*; **Neves, A. R.**; Correia-da-Silva, M.; Sousa, E.; Pinto, M. Carboxymethylation of flavonoids: synthesis, purification and structure elucidation. 8º *Encontro de Jovens Investigadores da Universidade do Porto* (IJUP15), 2015, p. 308.

Paper in preparation:

1. **Neves, A. R.**; Correia-da-Silva, M.; Sousa, E.; Pinto, M., The development of multitarget inhibitors to overcome the limitations of antithrombotic therapy: a practical overview on structure-activity relationships, *to be submitted to Current Drug Targets*.

* Presenting author

If you want to go fast go alone,

If you want to go far go together.

African Proverb

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ABSTRACT

According to World Health Organization, cardiovascular diseases are the first cause of death worldwide. Although health improved in the last decades, lifestyle changes led to an increased incidence of cardiovascular diseases. Currently, the available antithrombotic drugs are associated with significant drawbacks that limit their use and the development of more advantageous drugs with less secondary effects is necessary. A new class of polysulfated small-molecules with anticoagulant and antiplatelet activities was discovered in LQOF. However, these polysulfated derivatives showed poor antithrombotic efficacy by *in vivo* oral administration in mice, predicted to be due to poor absorption in the GI tract. The main aim of this work was to improve the oral bioavailability of these compounds. In order to get new optimized analogues two strategies were considered: i) obtaining conjugates with bile acids and ii) introduction of a triazole ring.

In this dissertation sixteen compounds were synthesized, ten of which were obtained and characterized for the first time, including three sulfated derivatives.

Naringin-deoxycholic acid conjugate **15** was obtained through a crosslinking reaction using TBTU as coupling reagent. Triazole linked xanthone glycoside **21** was obtained through a copper(I)-catalyzed alkyne-azide cycloaddition following by *O*- and *N*-deacetylation. Sulfation was successfully achieved with triethylamine-sulfur trioxide adduct under microwave irradiation.

Some intermediates (compounds **6**, **24**, and **26**) were tested for cell growth inhibitory activity. Rutin peracetate (**26**) showed good GI_{50} on six human tumor cell lines in the micromolar range.

The three sulfated derivatives (compounds **16**, **22**, and **27**) were screened for anticoagulant activity using the three classic clotting times APTT, PT, and TT. All the sulfated compounds prolonged the clotting times, and the most active compound was persulfated naringin-deoxycholic acid conjugate **16** exhibiting an $APTT_2$ in the micromolar range (44.2 $\pm$ 0.2 μM). These new optimized analogues with anticoagulant activity are expected to cross the GI tract membranes after oral administration.

Keywords: polyphenols; naringin; anticoagulant activity; oral bioavailability

RESUMO

Segundo a Organização Mundial de Saúde as doenças cardiovasculares são a principal cause de morte em todo o mundo. Apesar de ter havido um aumento na qualidade da saúde nos últimos anos, os hábitos de vida têm-se modificado o que levou ao aumento da incidência destas doenças. Atualmente, os fármacos antitrombóticos disponíveis estão associados a desvantagens que limitam o seu uso e é necessária a descoberta de melhores fármacos com menos efeitos secundários. No LQOF foi descoberta uma nova classe de pequenas moléculas polissulfatadas com atividade anticoagulante e antiagregante plaquetária. No entanto, estas moléculas demonstraram baixa eficácia antitrombótica após administração oral em ratinhos *in vivo* comportamento associado a baixa absorção no trato intestinal. O principal objetivo desta dissertação foi melhorar a biodisponibilidade oral desta nova classe de pequenas moléculas polissulfatadas. Duas estratégias foram aplicadas no sentido de se atingir o objetivo proposto: i) conjugação com ácidos biliares e ii) introdução de um anel triazole.

Nesta dissertação foram sintetizados dezasseis compostos, dez dos quais foram descritos e caraterizados pela primeira vez, incluindo três compostos sulfatados.

O conjugado naringina-ácido desoxicólico **15** foi obtido através de uma reação de ligação cruzada aplicando o TBTU como reagente de acoplamento. A xantona glicosilada ligada pelo anel triazole **21** foi obtida através de uma cicloadição alcino-azida catalizada por cobre (I), seguida de *O*- e *N*-desacetilação. A sulfatação foi realizada em micro-ondas utilizando aducto de trióxido de enxofre e trietilamina como reagente de sulfatação.

Alguns intermediários acetilados (compostos **6**, **24** e **26**) foram testados pela sua inibição do crescimento celular, tendo-se destacado a rutina peracetilada (**26**) como melhor composto, apresentando IG_{50} na ordem dos micromolar.

Os compostos sulfatados obtidos (compostos **16**, **22** e **27**) foram testados *in vitro* quanto à sua atividade anticoagulante através dos testes clássicos de coagulação APTT, PT e TT. Os três compostos testados prolongaram o tempo de coagulação e o conjugado sulfatado naringina-acido desoxicólico **16** foi o mais ativo, exibindo um $APTT_2$ na ordem dos micromolar (44,2 $\pm$ 0,2 µM).

É esperado que os novos análogos otimizados com atividade anticoagulante atravessem as membranas no trato gastrointestinal, após administração oral.

Palavras-chave: polifenóis; naringina; atividade anticoagulante; biodisponibilidade oral

ABBREVIATIONS

4-NPC - 4-Nitrophenyl chloroformate

APTT - Activated partial thromboplastin time

$APTT_2$ – Concentration required to double the activated partial thromboplastin time

brd - broad duplet

brs - broad singlet

d - duplet

DCC - *N,N'*-Dicyclohexylcarbodiimide

dd - double duplet

DMA - Dimethylacetamide

DMF - Dimethylformamide

DMSO - Dimethylsulfoxide

DOCA - Deoxycholic acid

DOCA-NH_2 - *N*-Deoxycholylethylamine

DOCA-NHS - Succinimido deoxycholate

EDA - Ethylenediamine

EDAC - 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide

EtOH - Ethanol

FA - Formamide

FXa - Factor Xa

GA - 18*β*-Glycyrrhetinic acid

GI – Gastrointestinal

GI_{50} – concentration required to inhibit growth by 50%.

HMBC - Heteronuclear multiple bond correlation

HRMS – High resolution mass spectrometry

HSQC - Heteronuclear single quantum correlation

IR - Infrared

J - Coupling constant

LMWH - Low molecular weight heparins

LQOF – Laboratório de Química Orgânica e Farmacêutica

m - multiplet

MCC - Mono-*N*-carboxymethyl chitosan

MeOH - Methanol

MW - Microwave

NaOAc - Sodium acetate

NHS - *N*-Hydroxysuccinimide

NMR - Nuclear magnetic resonance

PCP-Cys - Polycarbophil-cystein

PT - Prothrombin time

Py - Pyridine

q - quartet

s - singlet

SNAC - Sodium *N*-(8-(2-hydroxybenzoyl) amino) caprylate

SNAD - Sodium *N*-(10-(2-hydroxybenzoyl) amino) decanoate

SNOCC - *N*-Sulfonato-*N,O*-carboxymethylchitosan

t - triplet

TBTU - 2-(1*H*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro borate

THF - Tetrahydrofuran

TLC - Thin layer chromatography

TT - Thrombin time

UFH - Unfractioned heparin

OUTLINE OF THE DISSERTATION

The present dissertation is structured in six chapters:

INTRODUCTION

The first chapter includes a brief introduction of oral bioavailability of drugs, a state of the art concerning the strategies used to improve oral bioavailability of heparin. The aims of this dissertation are presented at the end of this chapter.

RESULTS AND DISCUSSION

In this chapter, results obtained from the synthesis, structure elucidation, and biological activity will be presented and discussed.

EXPERIMENTAL SECTION

In this chapter, the experimental procedures for the synthesis, structure characterization, and anticoagulant activity of the synthesized compounds are explained.

CONCLUSIONS

This chapter includes the main conclusions of the developed work.

REFERENCES

In this chapter, the references cited throughout the thesis are presented. The references followed the American Chemical Society style guide. The main bibliographic research motors were ISI Web of Knowledge, from Thomson Reuters, Scopus, PubMed and Google Scholar.

APPENDICES

The last chapter contains folding tables with spectroscopic data of the synthesized compounds.

CHAPTER 1 - INTRODUCTION

1.1. Oral bioavailability of drugs

In the last decades, the focus in the optimization of drug candidates has changed from improvement of potency to improvement of drug-like properties. Drug-like properties are defined as properties that drug candidates should have to become successful drug products.[1] Nowadays, drug-like properties are evaluated early, in the drug discovery pipeline, in order to eliminate compounds that could fail in clinical trials.

Oral administration is the most desired administration route and thus oral bioavailability plays an important role in drug discovery and development. Poor bioavailability by oral administration affects drug's efficacy and despite other administration routes can be chosen, this option should be avoided.

Several factors affect oral bioavailability of drugs, some being related to chemical structure of the drug (e.g. permeability, solubility) and others to the mechanisms developed by the organism to get rid of xenobiotics (e.g. first pass metabolism and efflux mechanisms).

In the gastrointestinal (GI) tract, drugs pass through biological membranes to reach the blood circulation using several mechanisms. Passive permeation is the mechanism more frequent in drug absorption and is related with solubility properties. Diverse strategies have been developed in order to surpass the poor oral absorption of drugs. Among these strategies are prodrugs, drug conjugates, structure optimisation and drug formulation.

Prodrug approach is one of the most successful strategy used to improve oral bioavailability of drugs and almost 10% of market drugs are considered prodrugs.[2] International Union of Applied Chemistry defines prodrug as a "drug containing specialized nontoxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule".[3, 4] Prodrugs are inactive compounds that need to be bioactivated to have activity. Activation is achieved through modification of the drug´s structure. This activation can be enzymatic or chemical before or after absorption.

Drug conjugates are emerging as a promising strategy to improve oral bioavailability of peptides and macromolecules that usually have large molecular weight and fail to be absorbed in the GI tract. [5-7] Drug conjugates have also been applied to low molecular weight drugs.[8, 9] Drug conjugates differ from prodrugs because they retain biological activity. Drugs can be conjugated with molecules which: i) will add lipophilic properties to the drug improving permeability through lipid membranes or ii) will be recognized by membrane transporters allowing the conjugate to cross the membrane through an active mechanism.

Deoxycholic acid (DOCA) and vitamin B_{12} have been used to conjugate with both large and low molecular weight drugs. DOCA has been conjugated with anticoagulants, antiviral, antifungal and antimicrobial drugs.[6-9] Vitamin B_{12} has been conjugated with antidiabetics

and anti-obesity drugs.[5, 7, 10] Drugs conjugated with these molecules will not pass the GI tract membranes through passive mechanisms as there are specific receptors to uptake bile acids and vitamins. DOCA is absorbed through apical sodium dependent bile acid transporter while vitamin B_{12} is absorbed by receptor-mediated endocytosis at intrinsic factor–B_{12} receptor in the apical region of enterocytes.[11, 12]

Structure optimization involves molecular modifications to improve pharmacokinetic properties and to increase the oral availability of drug candidates. There are a set of rules that predict if drugs are going to reach blood circulation successfully by passive permeation. The most known are the rules of five (Lipinski rules) and three.[13, 14] These rules were establish through the systematic study of properties of a great number of drugs that use passive permeation as the main mechanism to cross biological membranes in the GI tract and are widely used to predict oral availability in drug discovery and development.

Drug formulation regards to the use of excipients or micro and nanoparticles that will increase oral absorption of drugs. This strategy is applied mainly for poorly water-soluble drugs.

There are a great number of strategies to improve oral bioavailability of drugs that are applied in different stages and having different impacts on drug pharmacokinetic profiles.

1.2. Strategies to obtain orally-active heparins

Cardiovascular diseases are the leading cause of deaths in developed countries. New oral anticoagulants were recently introduced in the market, namely dabigatran (direct thrombin inhibitor), rivaroxaban, and apixaban - factor Xa (FXa) inhibitors -, but there are some hesitations about their wide use in the treatment of thromboembolic diseases.[15] These new anticoagulants seem to be more advantageous than the coumarinic oral anticoagulants. Although oraly-active, these drugs lack the polypharmacological actions of Heparin or unfractioned heparin (UFH) and low molecular weight heparins (LMWH) which are thought to be involved beyond the coagulation cascade[16], with antimetastic[17] and anti-inflammatory activities[18].

Heparin has been used in the clinic for more than 80 years and continues to be widely used in the treatment of thromboembolic events.[19] Heparin (**Figure 1**) is a mixture of highly sulfated glycosaminoglycans with a molecular weight between 5–30 kDa and is one of the most negative charged molecules in Nature.[20-22] Heparin is an indirect inhibitor of FXa and thrombin. LMWH (**Figure 1**) are also mixtures of glycosaminoglycans but with mean molecular weight of about 5 kDa.[23] LMWH derive from heparin by chemical or enzymatic depolymerization and have a more predictable pharmacokinetic profile.[24]

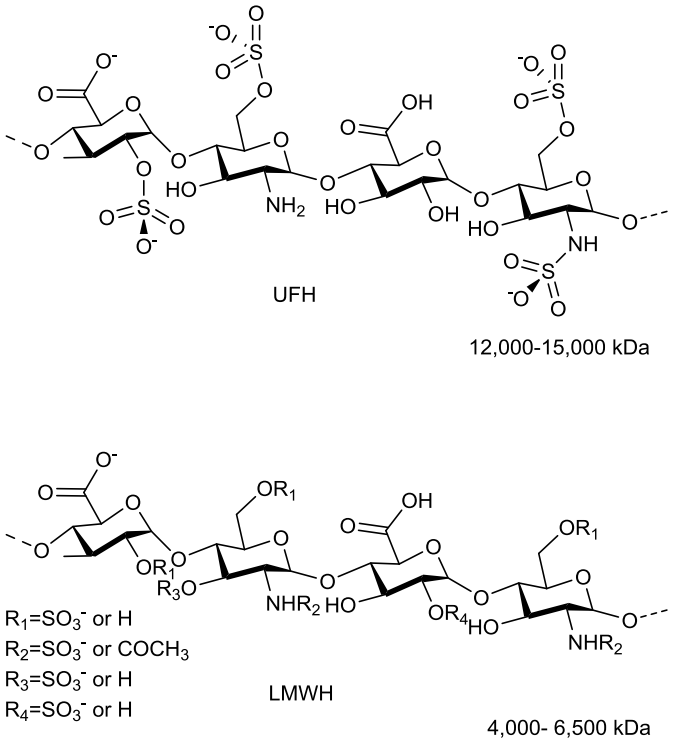


Figure 1 – Unfractioned heparin (UFH) and low molecular weight heparins (LMWH).

Compared with warfarin, the most prescribed oral anticoagulant[25], heparins have several advantages that surpass the fact that they are administered intravenously (**Figure 2**).[26, 27]

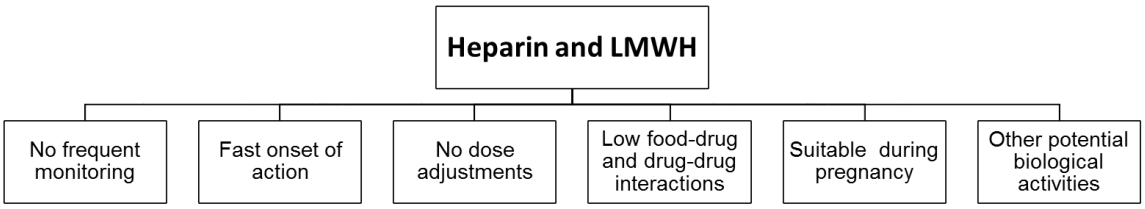


Figure 2 - Advantages of heparins over other anticoagulant drugs.

LMWH can be administered subcutaneously while UFH is administered intravenously, [23] however none exhibit oral bioavailability due to their highly negative charge, large molecular weight,[28] and rapid metabolism in the GI tract (**Figure 3**).[29]

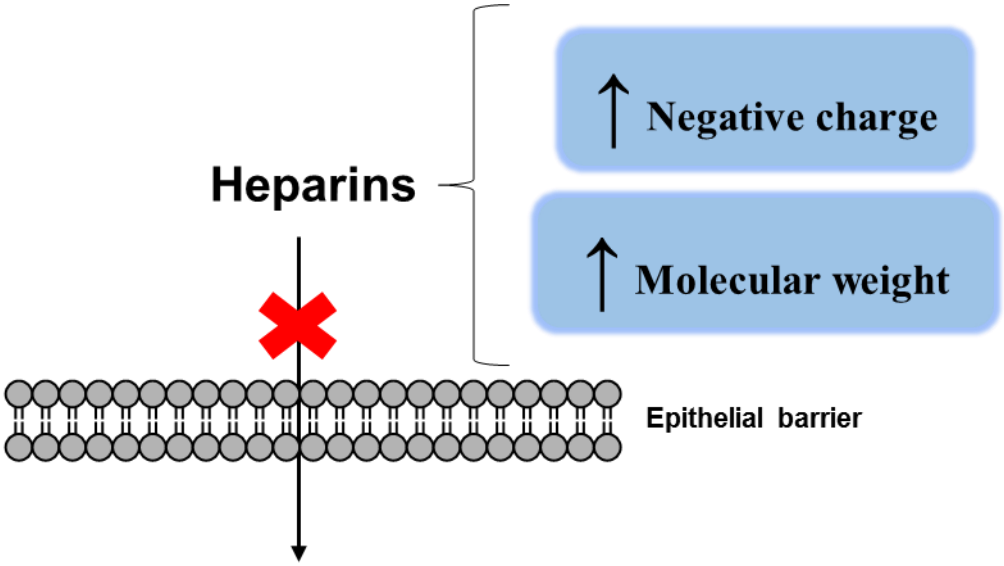


Figure 3 - Structural features of heparins that limit oral bioavailability.

There have been attempts to find suitable strategies that promote the absorption of heparin in the GI tract[30] and these can be divided in three main categories: drug conjugates, formulation with penetration enhancers, and micro and nanoparticle formulations.

1.2.1. Drug conjugates

Following a drug conjugate strategy, heparin has been covalently bond to other molecules in order to achieve oral bioavailability in one of two ways: increasing lipophilicity and permeability or enabling absorption via transporter proteins or receptor mediated endocytosis.[31]

There are some small-molecules that have been proven to be suitable choices for conjugation with heparin: DOCA and lipids.

1.2.1.1. Heparin-deoxycholic acid conjugates

DOCA (**Figure 4**) is a secondary bile acid produced from cholesterol and one of its functions is to stimulate the absorption of lipophilic molecules in the intestine.[32]

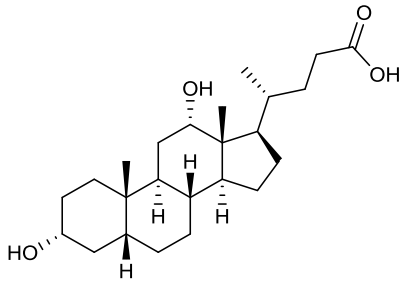
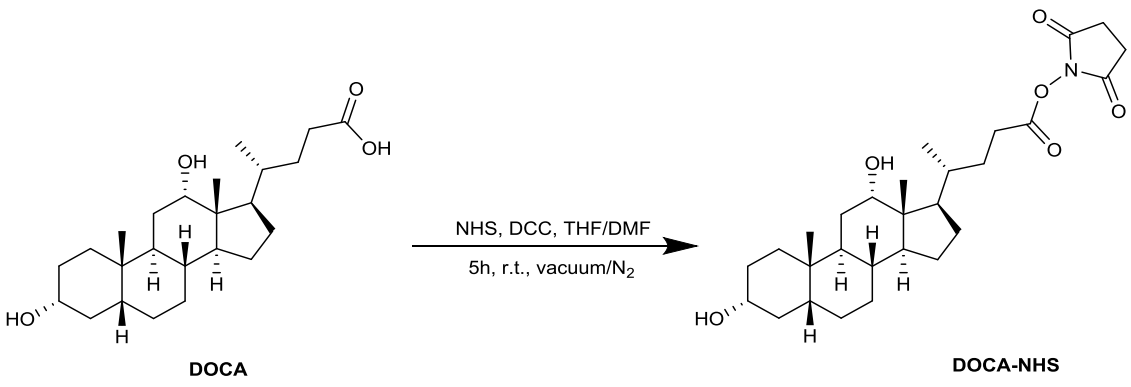


Figure 4 – Deoxycholic acid (DOCA).

DOCA is absorbed in the intestinal membrane through bile acid transporters and this mechanism of absorption would also increase the transcellular absorption of heparin.[6] Studies proved that heparin-DOCA conjugates increased the intestinal absorption of heparin, thus increasing its oral bioavailability.[6, 33, 34] Anticoagulant activity of heparin is conserved in the conjugate. DOCA is a naturally-occurring substance, so its oral administration may involve few toxic effects.[35] In fact, Lee, *et al.* carried out histological examination of the membrane of the GI tissue after oral administration of heparin-DOCA, and no damage to the microvilli and the cell layer was observed.[35]

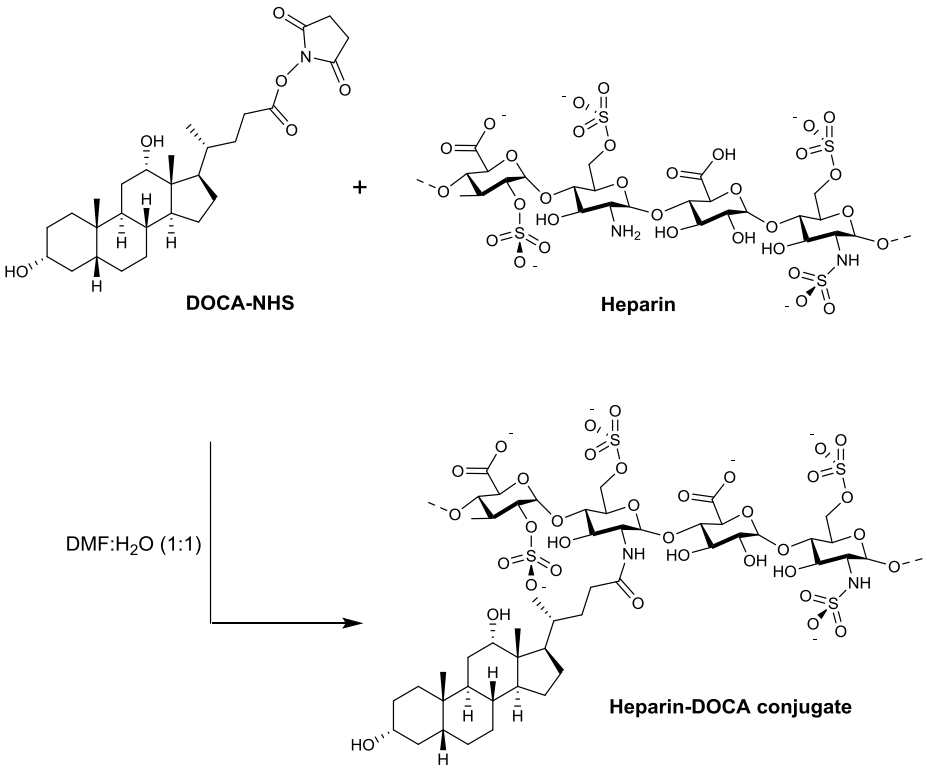
DOCA was conjugated with heparin using several synthetic approaches, although all of them involve the formation of an amide bond that will be cleaved after absorption.

To perform direct conjugation of the carboxylic acid of DOCA with the amine groups of heparin, carboxylic acid of DOCA has to be activated (**Scheme 1**).[33, 36]



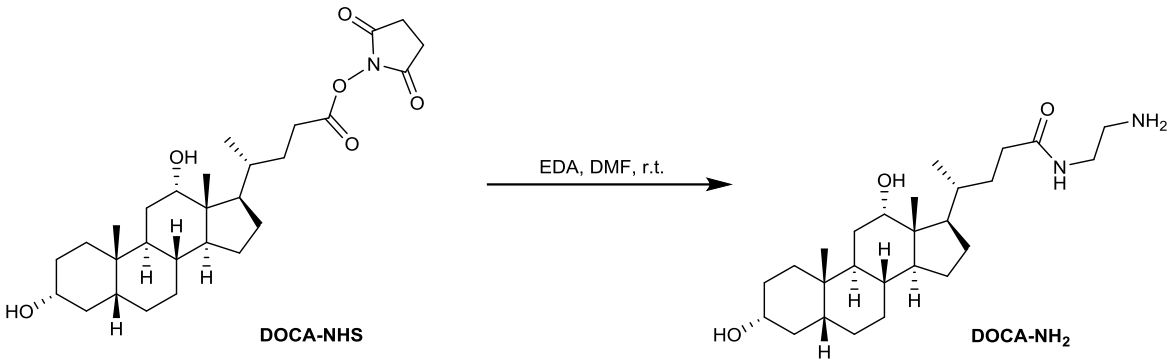
Scheme 1 - Activation of DOCA. NHS - *N*-hydroxysuccinimide, DCC - *N*,*N*-dicyclohexylcarbodiimide; THF - tetrahydrofuran; DMF – dimethylformamide.

After activation, succinimido deoxycholate (DOCA-NHS) reacts instantaneously with heparin to form the conjugate (**Scheme 2**).



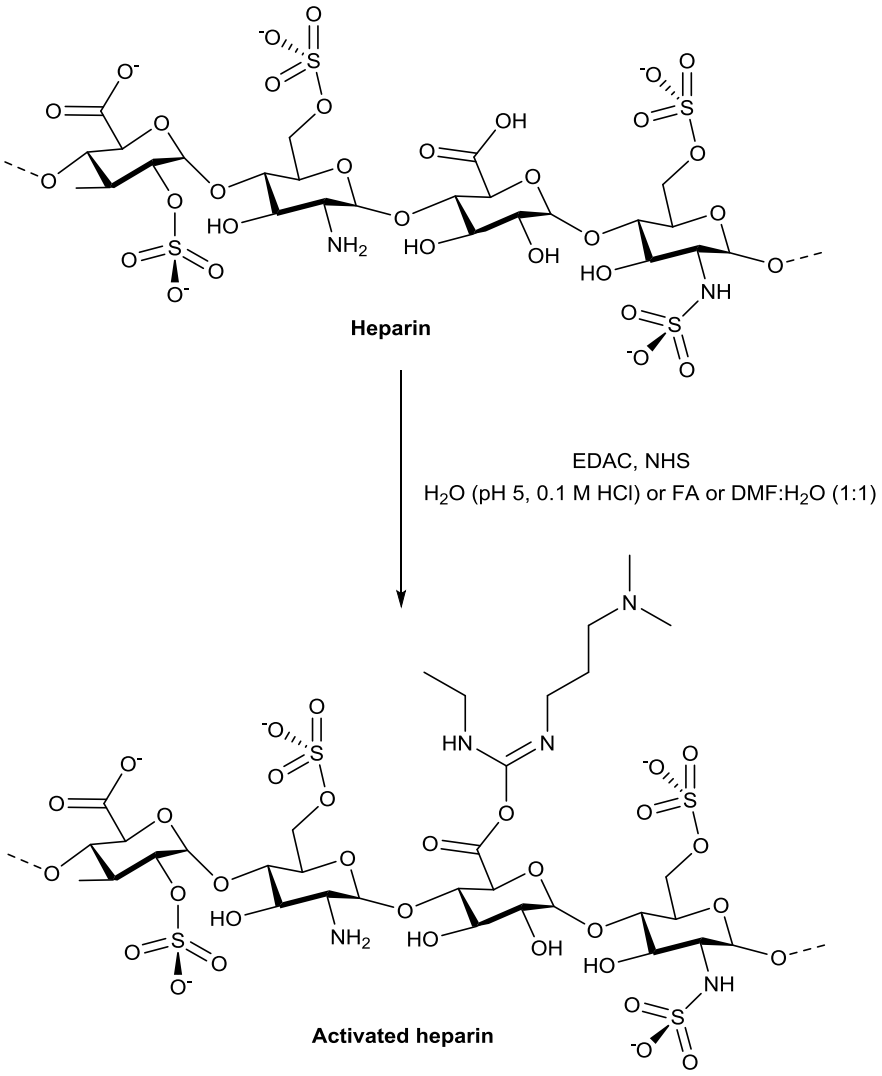
Scheme 2 - Direct conjugation of heparin and DOCA-NHS. DMF – dimethylformamide.

Another synthetic approach involves the coupling of the carboxylic acid of heparin and the primary amine of *N*-deoxycholylethylenediamine (DOCA-NH_2) previously prepared. DOCA-NH_2 is obtained through the reaction of DOCA-NHS with ethylenediamine (EDA) (**Scheme 3**).



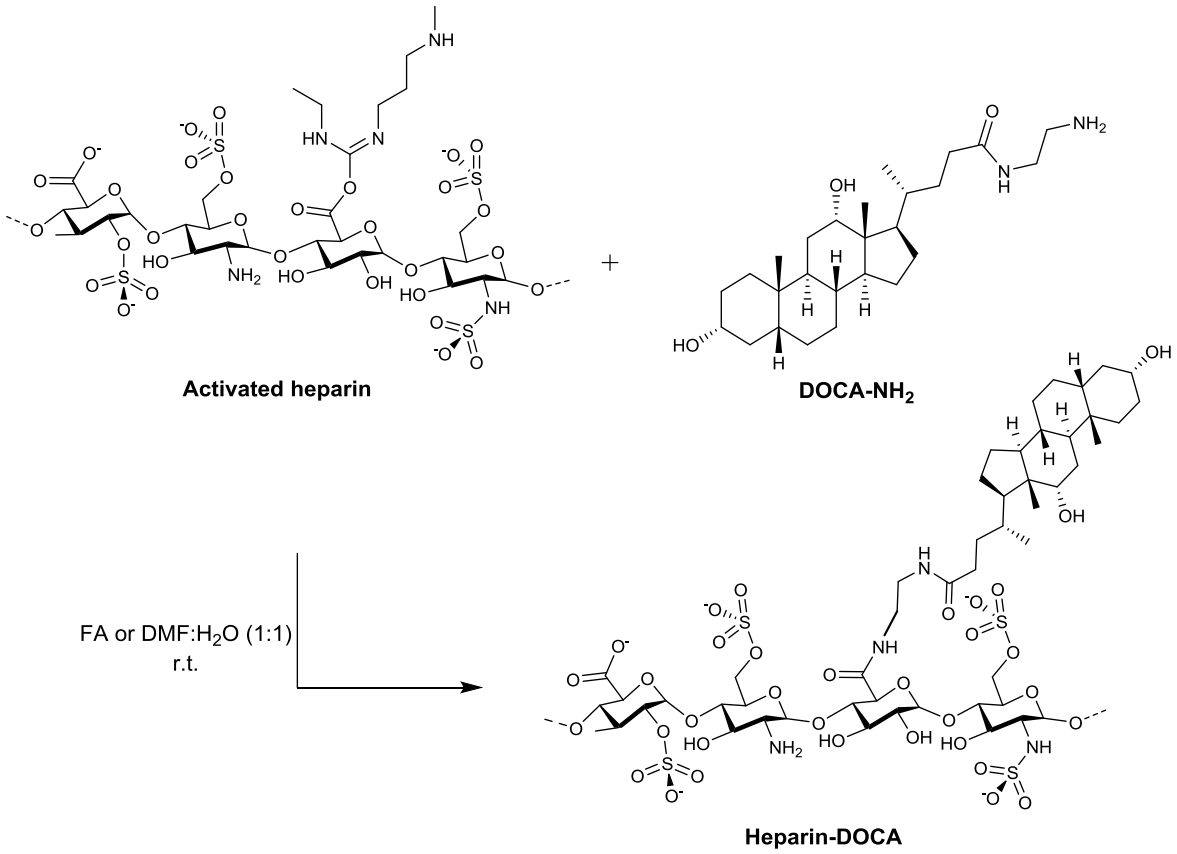
Scheme 3 - Reaction of DOCA-NHS with ethylenodiamine (EDA). DMF - dimethylformamide; r.t. - room temperature.

Activation of the carboxylic acid of heparin is performed before the conjugation reaction (**Scheme 4**). Generally, activating reagents used are 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) alone[6, 36-39] or in combination with *N*-hydroxysuccinimide (NHS)[40].



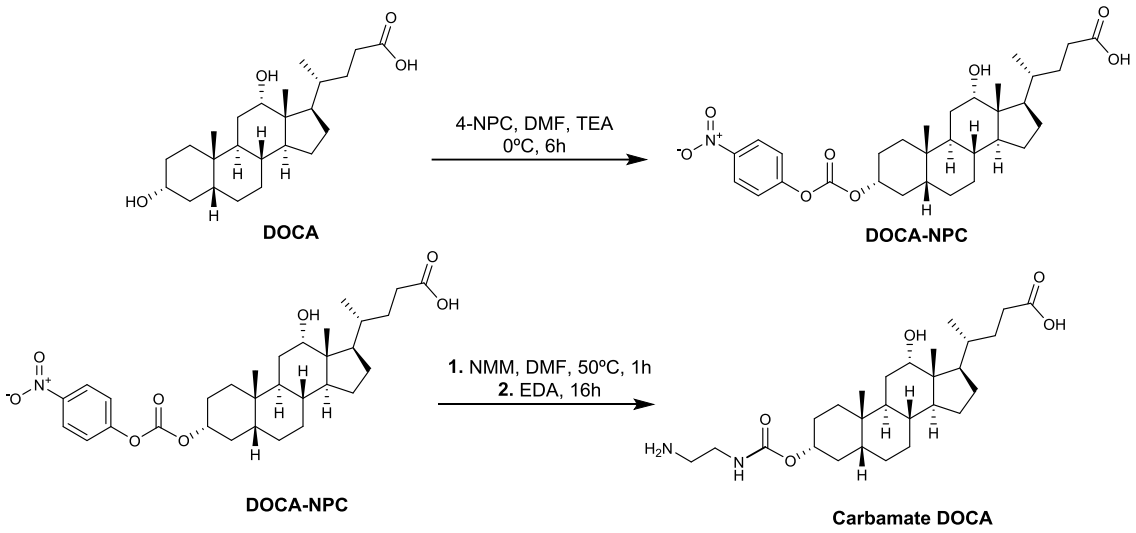
Scheme 4 - Activation of the carboxylic acid of heparin. EDAC - 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; NHS - *N*-hydroxysuccinimide; FA – formamide; DMF – dimethylformamide.

Activated heparin and DOCA-NH_2 react instantaneously as shown in **Scheme 5** to originate the heparin-DOCA conjugate.



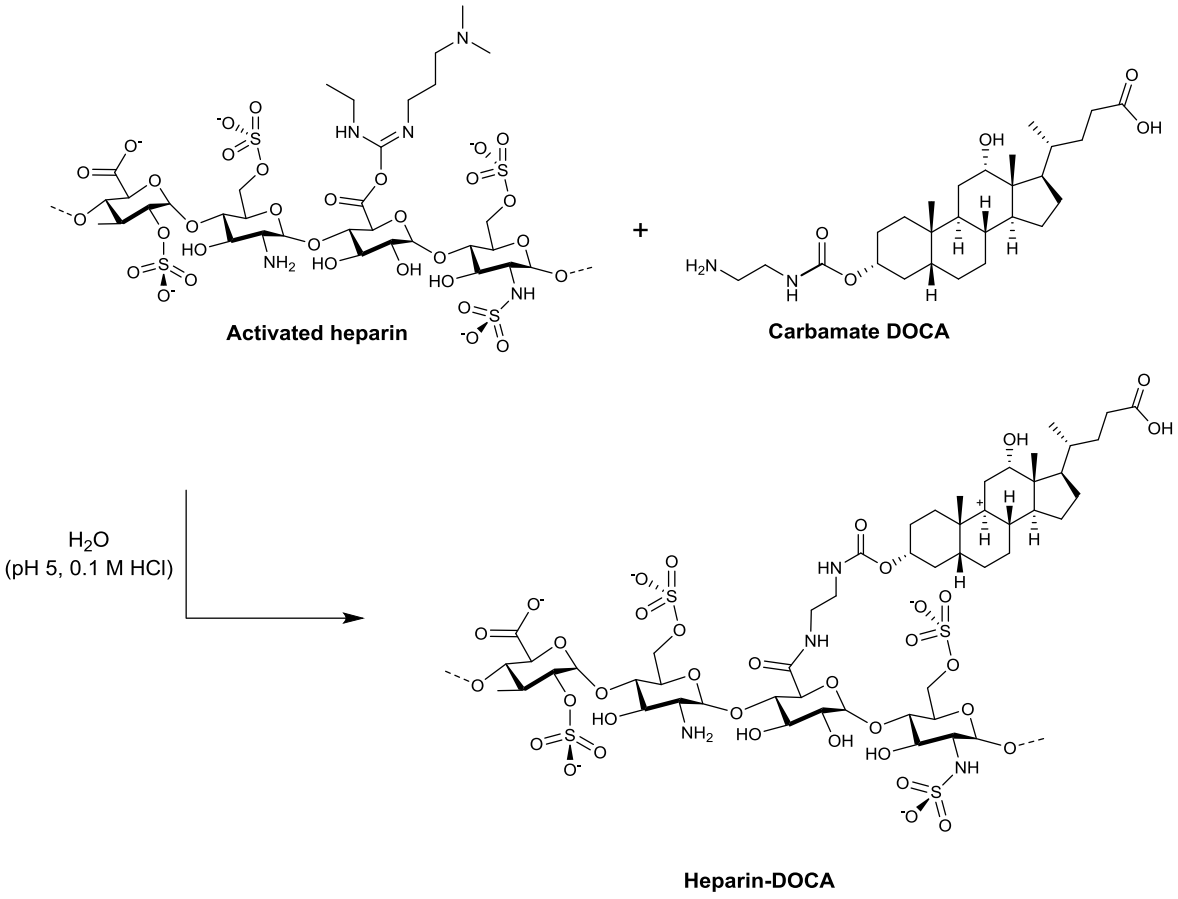
Scheme 5 - Reaction of activated heparin and DOCA-NH_2. FA - formamide; DMF - dimethylformamide; r.t. - room temperature.

Conjugation heparin with DOCA through the modification of an aliphatic hydroxyl group of DOCA was also described.[41, 42] This stratetegy consists in increasing the reactivity of 3-OH with the introduction of 4-nitrophenyl chloroformate (4-NPC) following reaction 4-methylmorphline and with EDA as shown in **Scheme 6**.



Scheme 6 - Synthesis of carbamate DOCA. 4-NPC - 4-nitrophenyl chloroformate; NMM – 4-methylmorpholine; EDA - ethylenediamine.

Carbamate DOCA further reacts with activated heparin as shown in **Scheme *7***.



Scheme 7 - Conjugation of heparin with carbamate DOCA.

1.2.1.2. Heparin-fatty acids and heparin-cholesterol conjugates

Fatty acids are carboxylic acids with a more or less long aliphatic chain, which make them amphiphilic compounds. They can be attached to other compounds, making them more lipophilic which, in the case of heparin, is useful in the attempt to enhance its absorption in the GI tract.

Lee *et al.* prepared fatty acids-heparin conjugates with palmitic acid and lauric acid (**Figure 5**).[35] The conjugates proved to retain anticoagulant activity and increased the absorption of heparin in rats, through the increase of lipophilicity. Palmitic acid and lauric acid were coupled with amine groups of heparin through their carboxylic groups. The strategy is the same used for heparin-DOCA conjugates.[35]

Paliwal *et al.* also synthesized fatty acids-heparin conjugates, with stearic acid, palmitic acid and myristic acid (**Figure 5**), coupling the carboxylic group of lipids with the amine groups of heparin.[43] As Lee *et al.*, they proved that these conjugates retained anticoagulant activity.

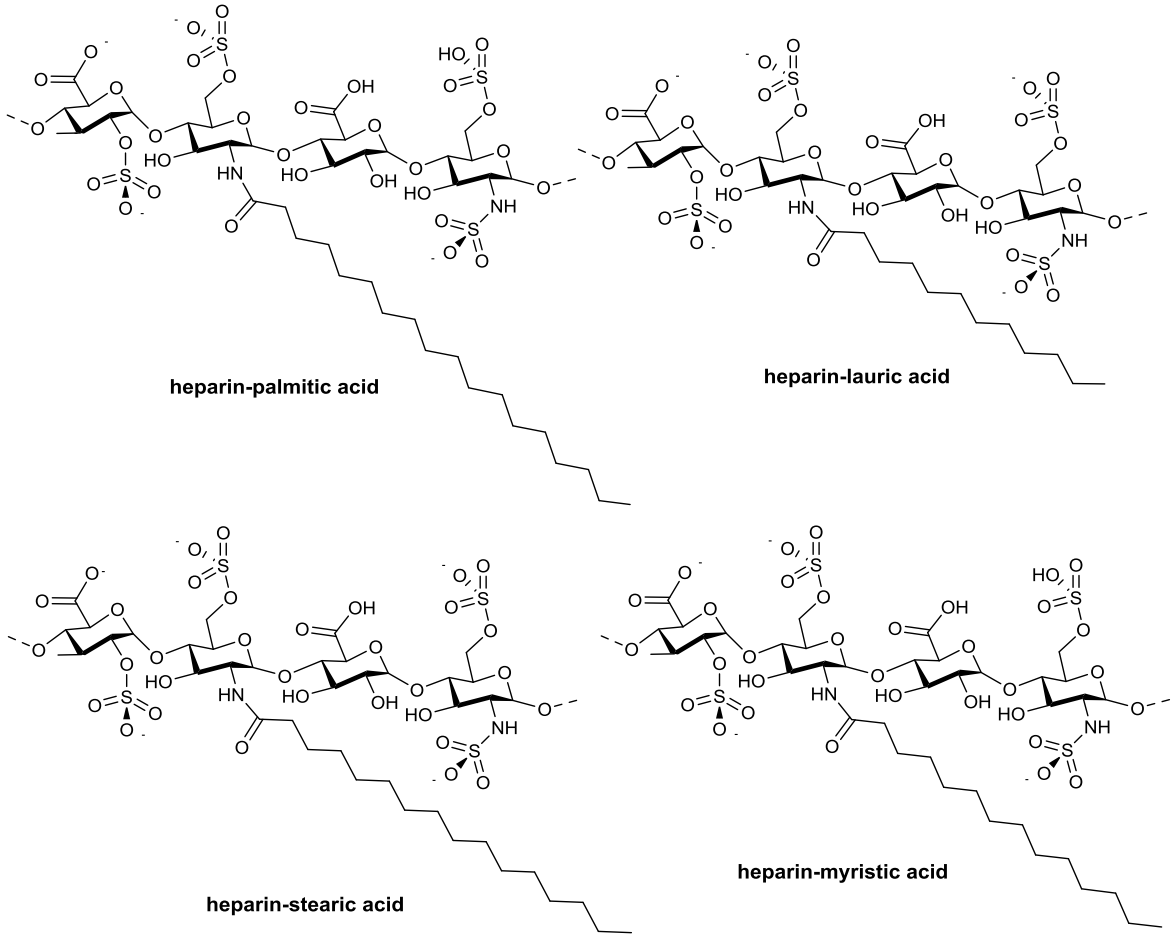
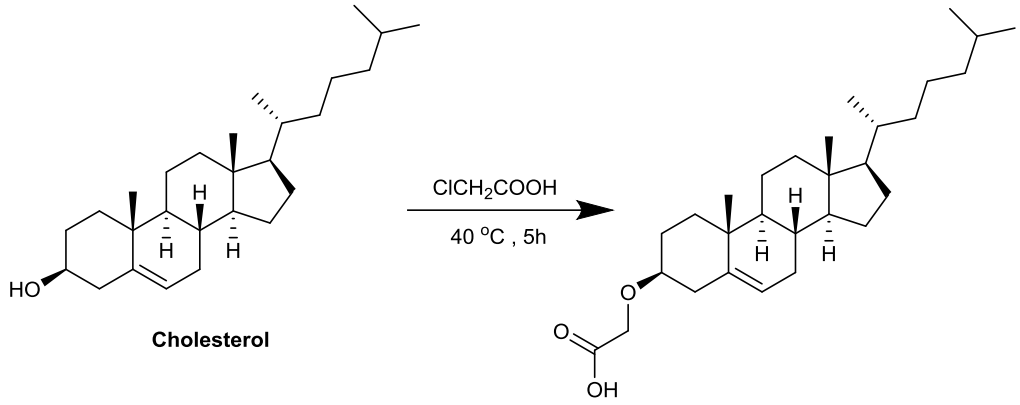


Figure 5 – Heparin-fatty acid conjugates.

Additionally, heparin was conjugated with cholesterol, a sterol that is present and plays an essential role in animal cell membranes. Nevertheless, heparin-DOCA conjugates showed higher prolonging effects than heparin-cholesterol conjugates.[35]

For the heparin–cholesterol conjugate, most of the experimental procedures were similar to those with heparin-DOCA. Amide coupling was preceded by the introduction of a carboxyl group. This was achieved by alkylation of the hydroxyl group, in which cholesterol reacted with chloroacetic acid (**Scheme 8**).[35]



Scheme 8 - Introduction of a carboxyl moiety in cholesterol.

In 2000,[35] a study showed that heparin-lipid conjugates, such as heparin-cholesterol, heparin-palmitic acid, and heparin-lauric acid conjugates had a lower absorption in the GI tract, compared with heparin-DOCA conjugate. These findings suggest that DOCA increases heparin absorption in the GI tract through receptor-mediated uptake.

1.2.2. Formulations with penetration enhancers

Penetration enhancers are compounds used to improve absorption of drugs in the GI tract either by opening the tight junctions between adjacent cells, enabling paracellular absorption - permeation enhancers - or by increasing the lipophilic properties of the drug enabling transcellular absorption - absorption enhancers (**Figure 6**).[31, 44]

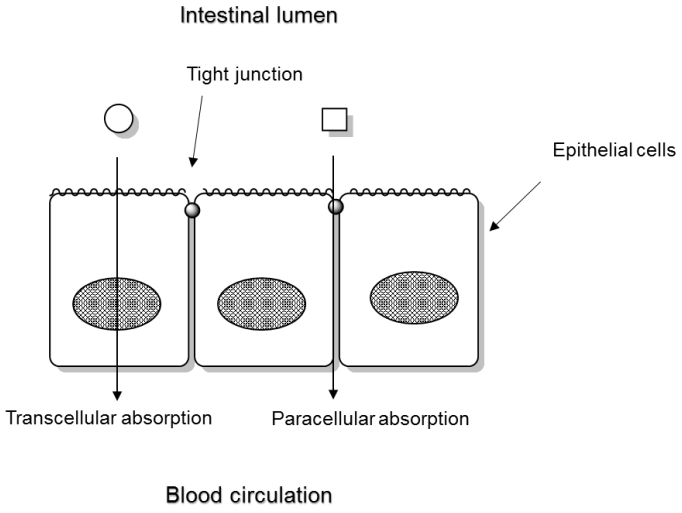


Figure 6 – Transcellular and paracellular absorption in the GI tract (adapted from [45]).

The weak association between enhancers and drugs allows the spontaneous release of the drug into the circulation.[46] However, many of the compounds examined *in vitro* as membrane permeation enhancers cause cytotoxicity or membrane damage. In addition, systemic toxic side effects of these compounds cannot be excluded.[47] Absorption enhancers are low molecular weight compounds that in contrast to permeation enhancers do not compromise the integrity of the intestinal epithelium.[46] In this section, some penetration enhancers that have already been used with heparin and LMWH will be revised.

1.2.2.1. Permeation enhancers

- **Sodium caprate**

Fatty acids can also be applied as permeation enhancers, enhancing the paracellular absorption of hydrophilic drugs at millimolar concentrations. Sodium caprate (**Figure 7**), the sodium salt of the aliphatic saturated 10-carbon fatty acid, capric acid, is the most comprehensively characterized medium length fatty acid as permeation enhancer.[46, 48] Oral delivery of LMWH using sodium caprate as penetration enhancer reached therapeutic levels and its cytotoxicity in Caco-2 cells was not found to be severe.[49]

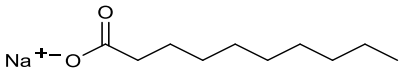


Figure 7 - Sodium caprate.

- **Glycyrrhetinic acid**

18*β*-Glycyrrhetinic acid (GA) (**Figure 8**) is a pentacyclic triterpenoid amyrin derivative obtained from the hydrolysis of glycyrrhizic acid, naturally present in the roots of the plant *Glycyrrhiza glabra*. GA was tested as a penetration enhancer in order to increase the intestinal absorption of LMWH by Motlekar *et al*..[50] Absorption of a LMWH was increased both *in vitro* and *in vivo* after co-administration with GA. After exposure to GA, no significant toxicity in Caco-2 cells monolayers was found, at relatively low concentrations. The authors hypothesised that GA could possibly be used as a penetration enhancer through paracellular absorption.[51] Although the study was conducted by Hisamitsu Pharmaceutical Co., Inc., the company does not seems to be developing a formulation of oral heparin with GA at the moment, being GA applied only in transdermal formulations.[52]

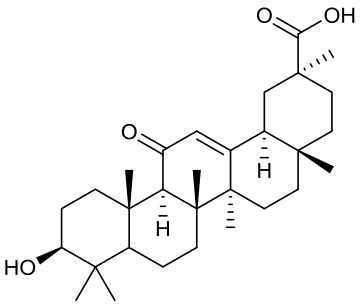


Figure 8 - 18*β*-Glycyrrhetinic acid.

Recently, anticoagulant properties through uncompetitive inhibition of FXa were described for GA.[53]

- **Mucoadhesive polymers**

Mucoadhesive polymers are polymers that have the capacity to adhere to mucous membranes. Chitosan derivatives and thiolated polymers will be addressed in this subsection as they increased the absorption of heparin or LMWH in the GI tract.

Chitosan is a polysaccharide with mucoadhesive properties that comprises glucosamine and *N*-acetylglucosamine subunits.[54] *In vitro* studies have shown that chitosan opens epithelial tight junctions in a concentration- and pH-dependent way.[54] However, chitosan was incompatible with LMWH[55] and chitosan derivatives were prepared.[56, 57]

Thanou *et al*. synthesized mono-*N*-carboxymethyl chitosan (MCC) (**Figure 9**), a polyampholyte chitosan derivative that has shown to increase paracellular absorption of LMWH *in vitro*, in Caco-2 cells monolayers.[56] MCC also increased the intestinal absorption of LMWH *in vivo* in rats. When the complex passes the intestinal barrier, it dissociates to yield the active macromolecule.

A sulfonate derivative of *N*,*O*-carboxymethyl chitosan (SNOCC) (**Figure 9**) increased the permeation and absorption of LMWH both *in vitro* in Caco-2 cells monolayer and *in vivo* in rats through intraduodenal administration.[57] SNOCC compared with others absorption enhancers has the advantage of not being absorbed in the GI tract, which is due to its high molecular weight.

Thiolated polymers or thiomers have improved mucoadhesion properties and permeation enhancing properties due to the thiol groups.[58] The presence of thiol groups offer the advantage to form disulfide bonds between these novel polymers and the mucus gel layer, which mimics natural mechanism of secreted mucus glycoproteins, which are also covalently anchored in the mucus layer by the formation of disulfide bonds.[59] Polycarbophil-cystein (PCP-Cys) (**Figure 9**) is a thiomer that was found to increase the absorption of

LMWH to anticoagulant levels.[58] The oral administration of heparin with PCP–Cys resulted in a significantly increased absorption of LMWH compared with control tablets comprising unmodified PCP or to an orally given aqueous heparin solution.[58]

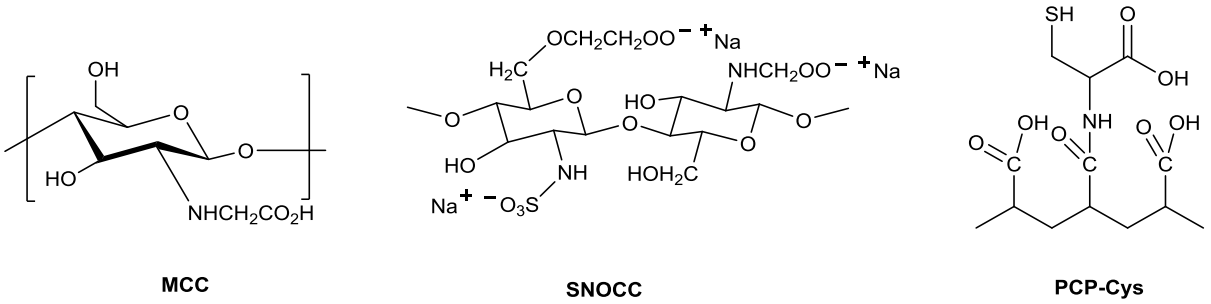


Figure 9 - Mono-*N*-carboxymethyl chitosan (MCC), a sulfonate derivative of *N*,*O*-carboxymethyl chitosan (SNOCC), and thiolated polycarbophil (PCP-Cys).

- **SNAC and SNAD**

Sodium *N*-(8 [2-hydroxybenzoyl] amino) caprylate (SNAD) and sodium *N*-[10-(2-hydroxybenzoyl) amino] decanoate) (SNAC) (**Figure 10**) interact non-covalently with heparin, neutralizing its negative ionic charge to render it more lipophilic.[44] SNAC increase the absorption of heparin through the GI tract in therapeutic doses.[60] Once the complex crosses the membrane through a paracellular route, SNAC dissociates from the therapeutic agent.[46, 61]

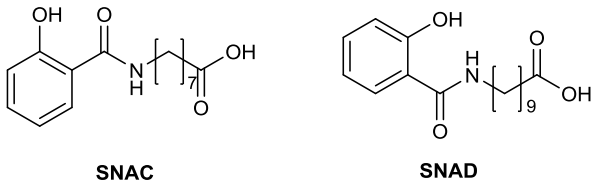


Figure 10 - Sodium *N*-(8 [2-hydroxybenzoyl] amino) caprylate (SNAC) and sodium *N*-[10-(2-hydroxybenzoyl) amino] decanoate (SNAD).

Oral heparin/SNAC entered clinical trials and showed good results in healthy volunteers and in patients undergoing elective total hip arthroplasty.[62] LMWH/SNAD has also shown to prevent deep venous thrombosis after oral route.[63] These studies demonstrated for the first time that heparins can be effectively orally delivered into the bloodstream in patients.[64]

However, there are still no formulations with orally-active heparin currently in the market.

1.2.2.2. Absorption enhancers

- **Polycationic lipophilic-core dendrons**

Polycationic lipophilic-core dendrons (**Figure 11**) are absorption enhancers that have proven to enhance absorption of LMWH in rats. They form lipophilic ion-pairs with the polyanionic LMWH making them hydrophobic. This ion-pair model of absorption assumes that the dendrons are absorbed as a complex with LMWH.[44] However, poor aqueous solubility of the complex dendron-LMWH limited its absorption.[65]

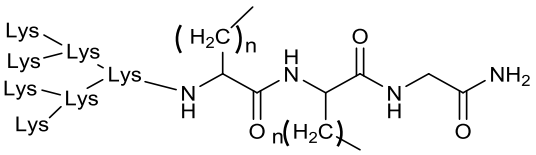


Figure 11 - Polycationic lipophilic-core dendrons.

- ***N*-Deoxycholylethylenediamine (DOCA-NH_2)**

Through lipophilic ion pairing, Lee, *et al.* designed a DOCA derivative, DOCA-NH_2, (see Introduction, **Scheme 3**) to complex with LMWH.[66] The complex was dissolved in propylene glycol and administered to rats by oral gavage. The physically association of DOCA-NH_2 with LMWH turn the later more lipophilic and *in vivo* experiments indicated that DOCA-NH_2 significantly affected the oral absorption of the LMWH, and at the molar ratio of 1:5 the oral absorption of LMWH was high. Although, the complex LMWH/DOCA-NH_2 has reduced solubility due to the larger size of the complex and propylene glycol had to be used as solubilizer. The oral absorption of LMWH/ DOCA-NH_2 complex was higher than those of LMWH/DOCA complex. The mechanism used to improve LMWH absorption is not clear and may be by bile acids transporters or passive absorption. However, no toxicological studies were performed and side effects cannot be excluded.

The use of penetration enhancers appears to be a good strategy to improve oral availability of heparin. An advantage is the preservation of the chemical structure of the drug.[67] However, toxic effects must be evaluated. Advantages and disadvantages of the penetration enhancers presented are illustrated in **Table 1**.

Table 1 - Advantages and disadvantages of penetration enhancers.

Penetration enhancer	Mechanism	Advantages	Disadvantages
Sodium caprate*	Paracellular absorption	No severe toxicity in Caco-2 cells ; increase absorption at millimolar range	Local toxicity
GA*	Paracellular absorption	No significant toxicity in Caco-2 cells monolayers after exposure to GA [50]	Not described
Chitosan derivatives (SNOCC and MCC)*	Increase the residence time in the GI tract and promote paracellular absorption of the drug	Biocompatible; not absorbed (elevated molecular weight) - few systemic side/toxic effects	Lower efficiency of MCC when compared with other delivery agents
Thiomers*	Paracellular absorption	Improved mucoadhesion properties and permeation enhancing properties due to the thiol groups	Possible toxic effects
SNAC and SNAD*	Paracellular absorption	Release the drug after absorption	Toxic effects (absorbed with the drug)
Polycationic lipophilic-core dendrons#	Transcellular absorption	Suitable for ionizable molecules	Absorbed with the drug (toxic effects); poor aqueous solubility of the complex dendron-LMWH
DOCA-NH_2#	Transcellular absorption	Recognition through bile acid transporters	Not described

***permeation enhancer; #absorption enhancer.**

1.2.3. Micro and nanotechnology

Micro and nanotechnology constitute two approaches widely used in drug delivery to enhance drug's pharmacokinetic profile and decrease its side effects.

Both polymeric micro and nanoparticles have been used to improve oral absorption of heparin.[68-72] Biodegradable polymers (poly-ε-caprolactone and poly-D,L-lactic-co-glycolic acid) and non-biodegradable polymers (Euparin dragit® RS and RL) were used to prepare heparin-loaded nanoparticles.[68, 69, 71, 72] It was observed that heparin maintained its anticoagulation activity, yet only *in vitro* studies were performed. These polymers were also used to prepare nanoparticles to encapsulate heparin.[70] Initial *in vitro* tests showed satisfactory encapsulation efficiency and controlled drug release with retention of the anticoagulant activity.[72] Further *in vivo* studies in rabbits showed oral absorption of heparin after administration.[68]

Later, LMWH (tinzaparin)-loaded nanoparticles were prepared using polyester and polycationic polymethacrylate.[71] Oral absorption of heparin was improved and its anticoagulant effect was prolonged for up to 8 hours.

It can be found in the literature a combination of strategies through the incorporation of lipid-heparin conjugates in solid lipid nanoparticles.[43] Incorporation of these conjugates into solid lipid nanoparticles significantly improved the bioavailability of LMWH after oral route administration with insignificant toxicity to different GI tissue.

Nevertheless, this approach has drawbacks that are related with the delay they create in drug absorption and the lack of control retained over absorption time as a result of the variability in intestinal motility and gastric emptying. Toxicity also has to be considered, as long as polymeric materials modify tight junctions and could lead to the absorption of endotoxins and other potentially toxic compounds.[73]

1.3. Aims: development of polysulfated small-molecules towards new oral antithrombotic agents

In order to discover new antithrombotic drugs with better pharmacokinetic profiles one strategy was adopted in Laboratório de Química Orgânica e Farmacêutica (LQOF): incorporate an oligosulfated moiety into a phenolic molecule (flavonoid and xanthone) in order to mimetize anticoagulant polysaccharides (**Figure 12**) while increasing the overall hydrophobicity.[74, 75] In contrast to UFH, polysulfated small-molecules have less charge density, reduced anionic character, higher hydrophobic nature, and have a defined composition and feasible synthesis.

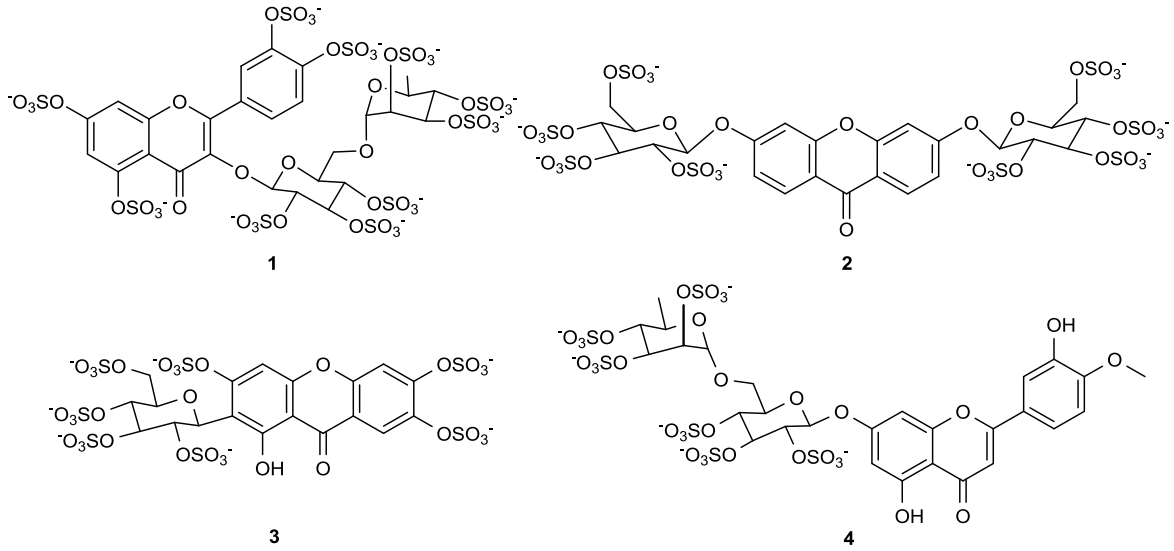


Figure 12 – Examples of polysulfated glycosidic flavonoids/xanthonoids: rutin persulfate (**1**), 3,6-(*O*-*β*-glucopyranosyl) xanthone persulfate (**2**), mangiferin heptasulfate (**3**) diosmin persulfate (**4**).

The anticoagulant activity of these sulfated compounds such as exemplified in **Figure 12** (compounds **1**-**4**) was evaluated *in vitro* by the classical clotting times. Polysulfated compounds **1**-**4** prolonged activated partial thromboplastin time (APTT)[74, 75] and the most potent compound (compound **2**) exhibited an $APPT_2$ (concentration required to double the activated partial thromboplastin time) in the micromolar range (ca 60 μM).[75] The prothrombin time (PT) or thrombin time (TT) pathways were less affected. After intraperitoneal administration in mice, polysulfated compounds revealed a systemic anticoagulant action with a rapid onset of action.[74, 75] Polysulfated small-molecules showed high solubility, stability in human plasma, and efficacy as antithrombotic, and preliminarily

in vivo toxicological studies in mice showed that these small-molecules are not expected to induce acute hepatic toxicity.[74, 75]

Furthermore, some of the synthesized polysulfated derivatives exhibited simultaneously anticoagulant and antiplatelet activities.[76] Even though a combined anticoagulant and antiplatelet therapy for patients with multiple disorders is still discussed, compounds combining in the same molecule anticoagulant and antiplatelet activities are believed to be promising drugs in preventing and/or treat both venous and arterial thrombosis. Dual inhibitors should be advantageous, due to expected less complex pharmacokinetics, lower incidence of side effects, and less demanding clinical studies.[77]

Nevertheless, after oral administration, none of the tested compounds was active.[78]

Thus, the main purpose of this dissertation was the improvement of oral bioavailability of these polysulfated small-molecules.

Two strategies were considered to achieve this purpose:

i) BILE ACID CONJUGATION

Conjugation of DOCA was chosen as a potential strategy to improve oral bioavailability of polysulfated small-molecules. DOCA is absorbed in the GI tract through specific membrane receptors and was successfully used to improve heparin bioavailability without significant toxicity (Section 1.2), in contrast to permeation enhancers. In fact, targeting membrane receptors is a strategy used for the oral delivery of drugs.[31] Fluconazole and acyclovir have already been successfully conjugated with molecules that are recognized by intestinal receptors to increase oral bioavailability.[8, 9]

ii) INTRODUCTION OF A TRIAZOLE

Introduction of a triazole in the polysulfated glycosidic small-molecules was planned as a strategy to increase lipophilicity. Triazole has been used as a strategy to improve lipophilicity of several types of drugs,[79-81] as antifungal and anti-human immunodeficiency virus drugs.[80, 81]

Thus, the specific aims were:

i) to synthesize new phenolic bile acid conjugates,

ii) to synthesize triazole-linked phenolic glucosides,

iii) to synthesize sulfated derivatives of the compounds referred in **i** and **ii**,

iv) to characterize through spectroscopic techniques the structure of the synthesized derivatives,

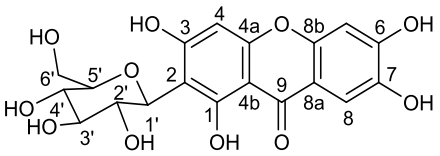
v) to screen the biological activities of the obtained compounds (**i**, **ii**, and **iii**).

CHAPTER 2 - RESULTS AND DISCUSSION

2.1. SYNTHESIS

2.1.1. Conjugation of mangiferin with deoxycholic acid

Mangiferin (**5**, **Figure 13**) is a naturally-occurring xanthone-*C*-glucoside found mainly in mango tree *Mangifera indica*. Compound **5** was the first xanthone to be investigated pharmacologically[82] and shows several biological activities, such as antioxidant[83-85], antidiabetic[86, 87], and antiviral.[88, 89] Derivatives of compound **5** such as acetyl[83, 90], propionyl[90, 91], butyryl[90], cinnamoyl,[83] benzyl,[92, 93], and substituted anilyl derivatives[94-96] also displaying a wide range of biological activities were described.



5

Figure 13 - Mangiferin (**5**).

In LQOF, mangiferin heptasulfate (**3**, **Figure 12**) was synthesized and in *in vitro* studies showed anticoagulant activity.[75] However, in *in vivo* studies mangiferin heptasulfate (**3**) was not active after oral administration.[75] Permeability studies using Ussing chamber verified that the compound was not able to cross the intestinal membrane (unpublished results).

Taking these results in account, conjugation with DOCA was planned in order to increase intestinal absorption.

2.1.1.1. Acetylation of mangiferin (5)

Compound **5** has four phenolic groups with the following reactivity order: 3, 6, and 7-OH > 1-OH.[97] So, it was planned to protect the phenolic groups of mangiferin except 1-OH, to be available for further conjugation with DOCA.

Acetylation is a commonly used reaction to protect functional groups, because of simple deprotection and stability of the groups in mild basic or acid conditions. To obtain the acetylated derivative of compound **5** with only 1-OH free, several conditions were attempt (**Table 2**).

Table 2 – Tested conditions for acetylation of compound **5**.

Entry	Conditions	Results
1	Ac_2O, Py, CH_2Cl_2, r.t.[98]	Several partially acetylated products were obtained.
2	Ac_2O/AcOH, NaOAc, reflux[99]	Several partially acetylated products were obtained.
3	Ac_2O, r.t.	No product formed
4	Ac_2O, NaF, MW, reflux[100]	Several partially acetylated products were obtained.
5	Ac_2O, I_2, MW, reflux[101]	Mangiferin peracetate was obtained.
6	Ac_2O, reflux[102]	Two principal products were obtained: mangiferin hepta- and peracetate

Ac_2O – acetic anhydride; MW – microwave; NaOAc – sodium acetate; Py – pyridine; r.t. – room temperature; AcOH – acetic acid.

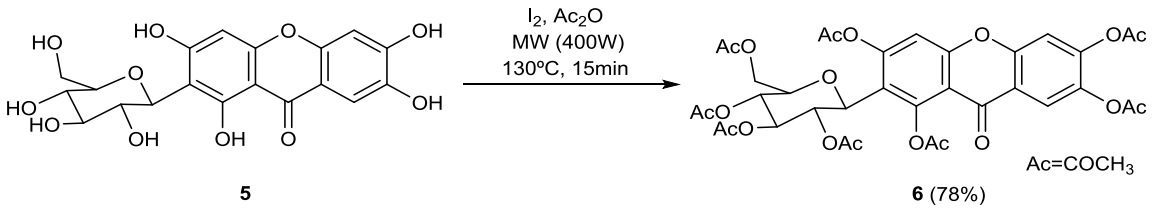
Acetic anhydride was the selected reagent to introduce acetyl groups due to its wide use in acetylation.[21, 101, 103-105]

Since acetylation can be catalysed by bases, acids, metallic and non-metallic Lewis acids[105-107], pyridine was firstly chosen as the nucleophilic catalyst (**Table 2**, entry 1). Nonetheless, the reaction resulted in several partially acetylated products (**Table 2**, entry 1).

Other bases are employed in acetylation as catalysts, such as NaOAc (sodium acetate)·$3H_2O$.[103] Thus, NaOAc·$3H_2O$ in a mixture of acetic anhydride and acetic acid was investigated (**Table 2**, entry 2). These conditions were unsuccessful providing also a complex mixture of partially acetylated products.

NaF, an inorganic salt, was found to be an efficient catalyst in acetylation of hydroxyl groups.[100] Ready availability and low cost are some advantages of NaF. When applied to mangiferin (**5**) in solvent-free conditions and under microwave (MW) irradiation, several partially acetylated products were also obtained (**Table 2**, entry 4).

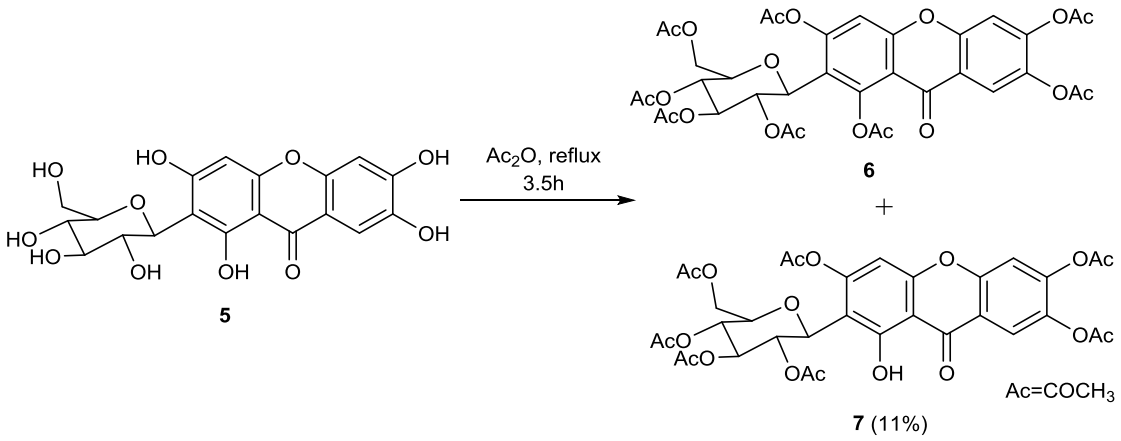
Molecular iodine is described as a strong and efficient catalyst for acetylation of alcohols, amines and phenols, being an inexpensive, non-toxic, non-metallic and readily available catalyst.[108, 109] When iodine was used as catalyst in the acetylation of mangiferin (**5**) in solvent-free conditions using an excess of acetic anhydride under MW irradiation (**Table 2**, entry 5; **Scheme 9**), this reaction condition gave one major product, mangiferin peracetate (**6**) in 78% yield.



5 **6** (78%)

Scheme 9 - Synthesis of mangiferin peracetate (**6**). Ac_2O - acetic anhydride; MW - microwave.

The derivative of compound **5** with the free 1-OH was only obtained after reflux with an excess of acetic anhydride (**Table 2**, entry 6; **Scheme 10**) in 11% yield.[102] Two products were obtained and only mangiferin heptaacetate (**7**) was isolated through flash chromatography column. Although the presence was detected by thin layer chromatography (TLC) by comparing with a standard, mangiferin peracetate (**6**) was not isolated.



5 **6** + **7** (11%)

Scheme 10 - Synthesis of mangiferin heptaacetate (**7**). Ac_2O – acetic anhydride.

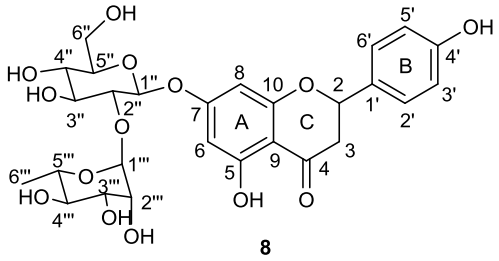
2.1.1.2. Carbomethoxymethylation of mangiferin (5)

The next synthetic step was the introduction of a carboxylic moiety at 1-position of compound **7** to increase reactivity for the following conjugation with DOCA.

Several conditions were attempt with compound **7**[110, 111] but all of them originated a complex mixture of compounds. A probably explanation for the formation of a mixture of products lies in the fact that acetyl groups may be replaced by alkyl groups.[110] Due to this, conjugation with DOCA was planned for another compound: naringin.

2.1.2. Conjugation of naringin with deoxycholic acid (I)

Naringin (**8**, **Figure 14**) is a flavanone glycoside naturally present in grapefruit and other citrus fruits. Similar to other natural flavonoids, compound **8** has a wide variety of biological activities and is commercialized as an antioxidant supplement.[112-114] Supplementation with compound **8** extracted from citrus fruits has been proved to decrease plasma lipid concentrations and to improve the antioxidant mechanism system.[115, 116] Thus, naringin (**8**) is a suitable model to plan potential antithrombotic derivatives.



8

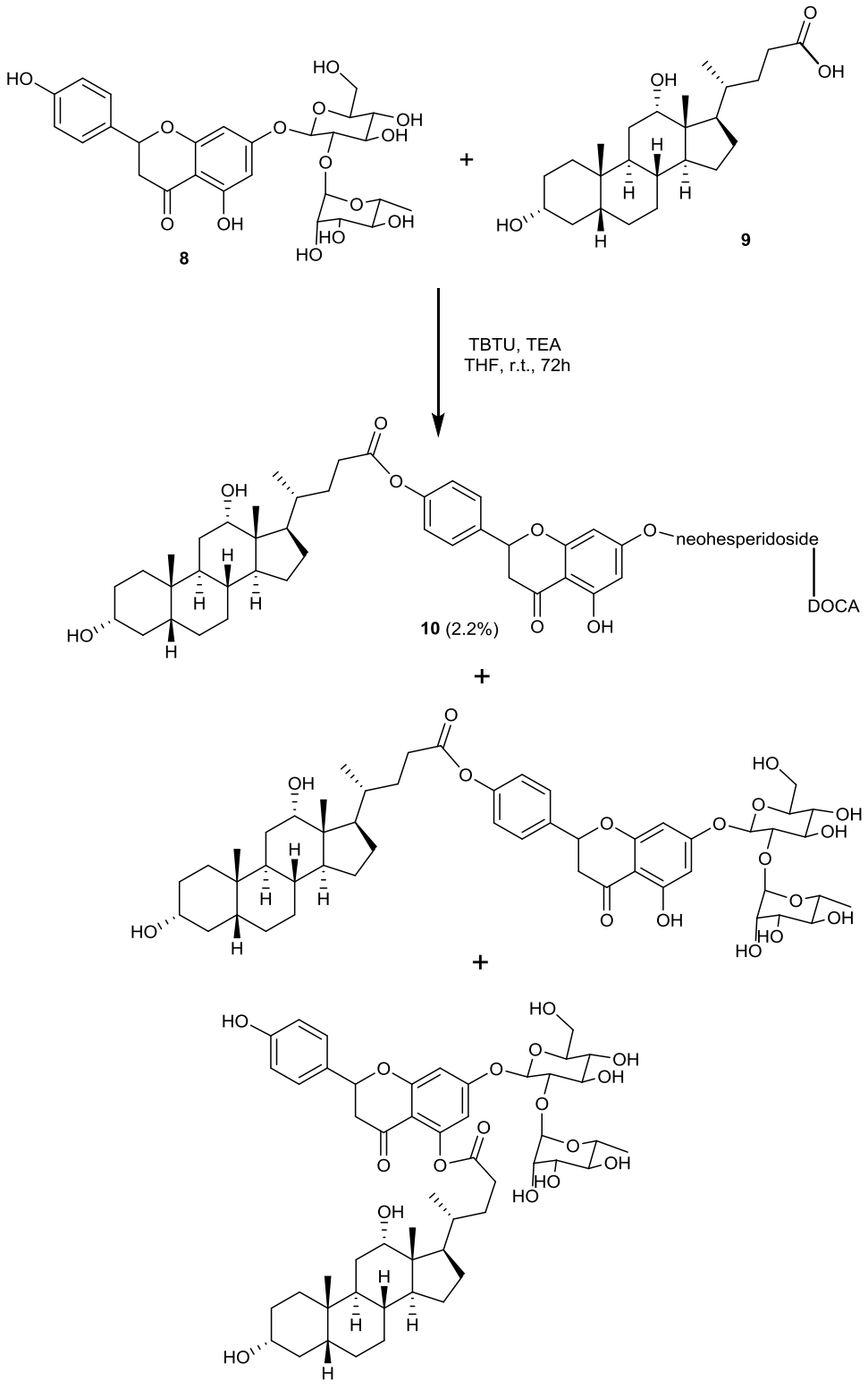
Figure 14 – Naringin (**8**).

Compound **8** was selected to conjugate with DOCA due to the well-established differences in the reactivity of the two phenolic groups present at 5 and 4' positions: 4'-OH is more reactive, while 5-OH is less available due to a hydrogen interaction with the C=O at C-4.

Due to the presence of a single highly reactive phenolic group (4'-OH), direct conjugation of compound **8** with DOCA (**9**) was firstly attempt.

Esterification of the carboxylic acid of DOCA (**9**) with the 4'-OH phenolic group of compound **8** was investigated using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro borate (TBTU) as the coupling reagent (**Scheme 11**). Esterification of carboxylic acids with phenols is easily accessible using TBTU in the presence of triethylamine (TEA), in smooth conditions and good yields.[117]

The coupling reaction is depicted in **Scheme 11**. Three products were detected but only compound **10** was isolated by flash chromatography column following preparative TLC in 2.2% yield. Spectroscopic data did not allow to confirm the exactly position of the second molecule of DOCA. The other two compounds were hypothesized to be monoconjugates of compound **8** with DOCA.



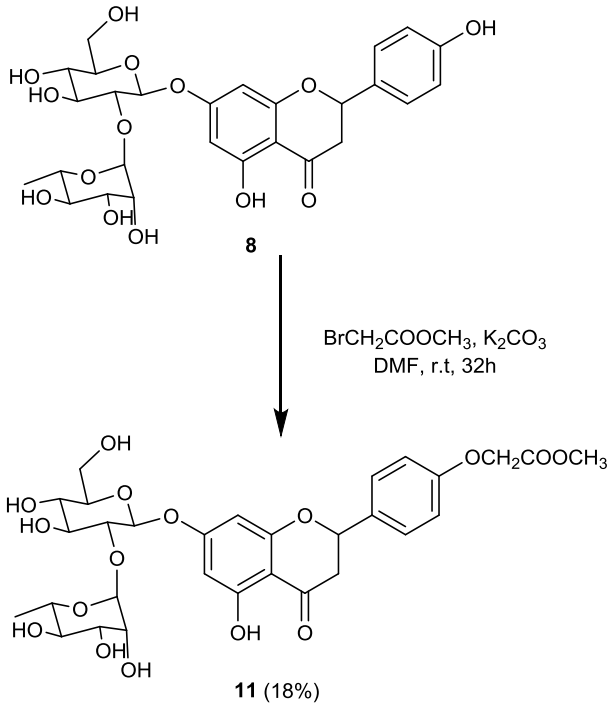
Scheme 11 - Synthesis of naringin-di-deoxycholate (**10**). TBTU - 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro borate; TEA – triethylamine; THF – tetrahydrofuran; r.t. - room temperature.

2.1.3. Conjugation of naringin with deoxycholic acid (II)

2.1.3.1. Carbomethoxymethylation of naringin (8)

Due to the results obtained in the direct conjugation of naringin (**8**) with DOCA (**9**), carbomethoxymethylation of 4'-OH was performed in order to increase reactivity in this position.

Methyl 4´-naringin acetate (**11**) was obtained within 32 hours in 18% yield (**Scheme 12**).

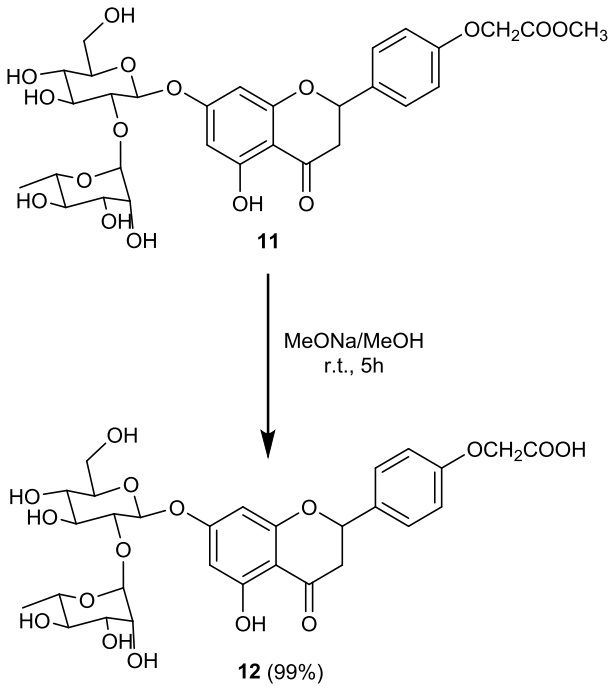


Scheme 12 - Synthesis of methyl 4'-naringin acetate (**11**). DMF - dimethylformamide; r.t. - room temperature.

2.1.3.2. Deacetylation of methyl 4'-naringin acetate (11)

4'-Naringin acetic acid (**12**) was obtained using a Zemplén deacetylation[123] with sodium methoxide in methanol (MeOH) at room temperature (**Scheme 13**). Zemplén deacetylation was selected because of the use of catalytic amounts of base (sodium methoxide), short reaction times, and excellent yields.[118]

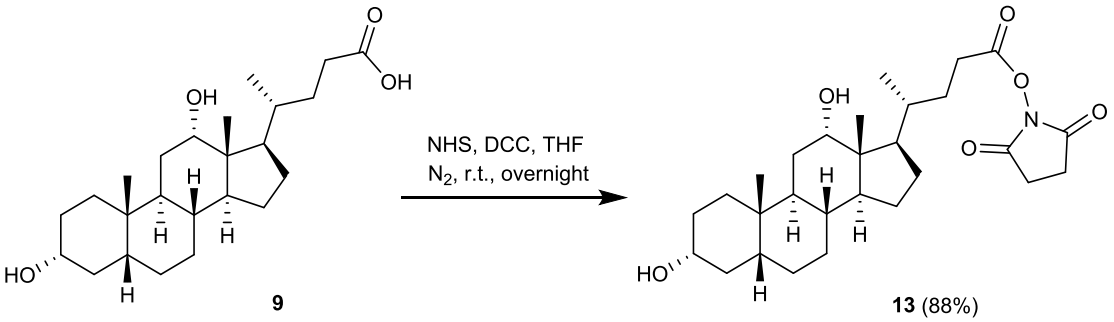
After the completion of the reaction neutralization using an ion exchange column was carried out to afford compound **12** in quantitative yields.



Scheme 13 - Synthesis of 4'-naringin acetic acid (**12**). MeONa - sodium methoxide; MeOH - methanol; r.t. - room temperature.

2.1.3.3. Synthesis of succinimido deoxycholate (13)

Before the conjugation of compound **12** with DOCA (**9**), activation of compound **9** was performed. Succinimido deoxycholate (DOCA-NHS, **13**) was synthesized in accordance with a method previously reported for the synthesis of heparin-DOCA conjugates (**Scheme 14**).[39] *N*,*N*-Dicyclohexylcarbodiimide (DCC) was used as the coupling reagent. DCC reacts firstly with the carboxylic acid of deoxycholic acid and is replaced by NHS to form the activated ester DOCA-NHS **13**, which is stable and can be isolated.

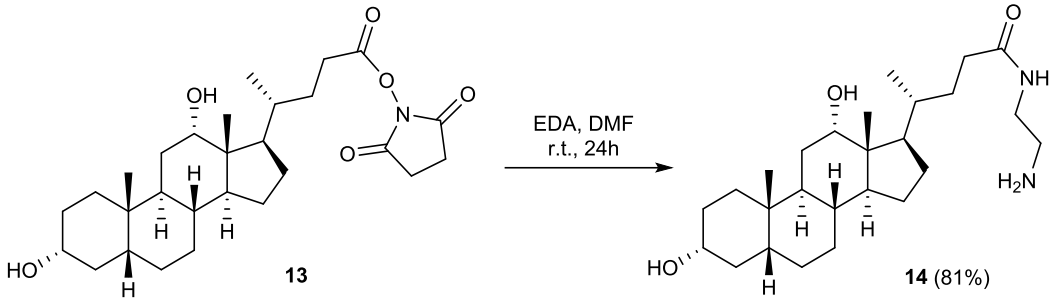


Scheme 14 - Synthesis of succinimido deoxycholate (DOCA-NHS, **13**). NHS - *N*-hydroxysuccinimide; DCC - *N*,*N*-dicyclohexylcarbodiimide; THF - tetrahydrofuran; r.t. - room temperature.

Purification was accomplished firstly by filtration of the by-product dicyclohexylurea and then by precipitation of the product from the reaction mixture. DOCA-NHS (**13**) was obtained in 88% yield.

2.1.3.4. Synthesis of *N*-deoxycholylethylenediamine (14)

The method employed to synthesize *N*-deoxycholylethylenediamine (DOCA-NH_2, **15**) was previously described in the synthesis of heparin-DOCA conjugates (**Scheme 15**).[6, 36-39]

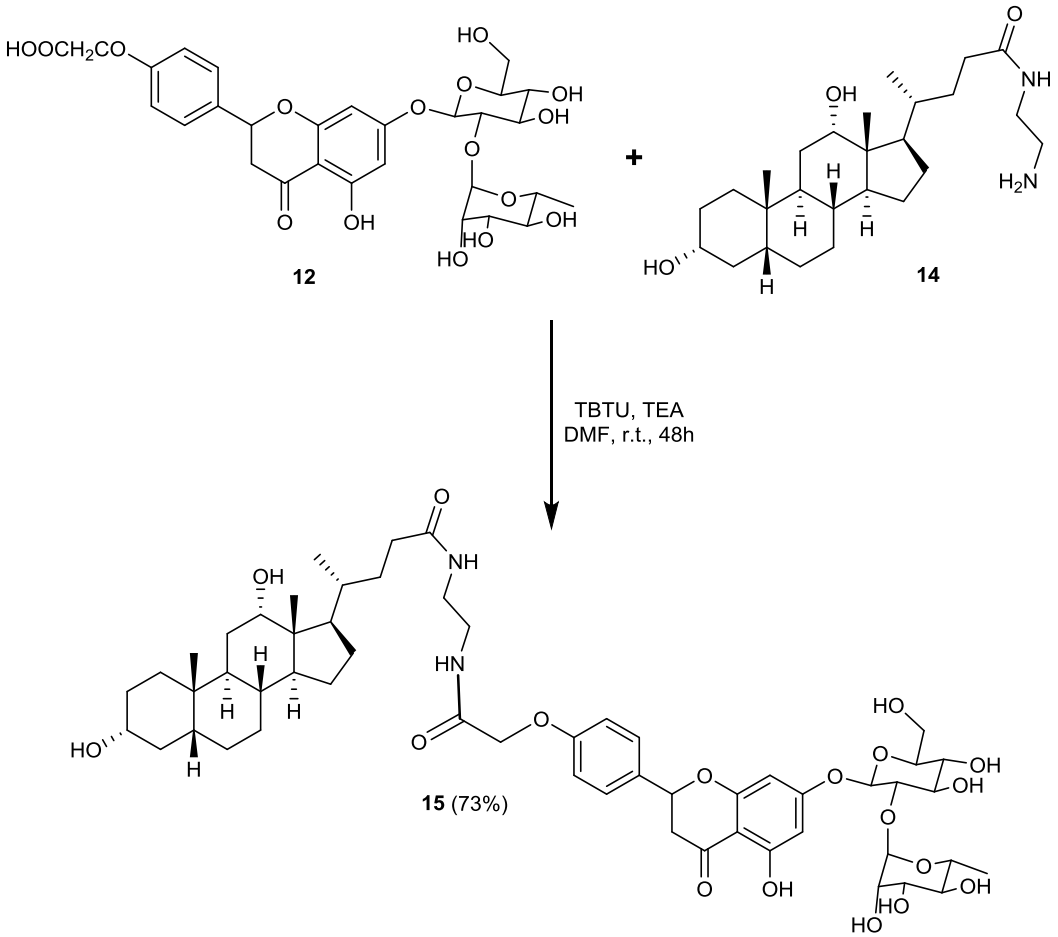


Scheme 15 - Synthesis of *N*-deoxycholylethylenediamine (DOCA-NH_2, **14**). EDA - ethylenediamine; DMF - dimethylformamide; r.t. - room temperature.

The product was obtained with 81% yield in 24 hours after pouring the reaction in ice following filtration, which allowed eliminating the unreacted EDA.

2.1.3.5. Synthesis of 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide (15)

A mixture of dimethylformamide (DMF) and H_2O is used as solvent in the synthesis of heparin-DOCA conjugates.[33-35] As heparin is highly soluble in H_2O, EDAC, a water-soluble coupling reagent, is extensively used for heparin-DOCA conjugation. Compound **12** was only soluble in the organic solvent DMF. So, in contrast to heparin methods, we used TBTU as the coupling reagent. TBTU was also selected because it was described to allow an efficient amidation of carboxylic acids with amines.[119] The reaction occurred at room temperature with the addition of a catalytic amount of TEA (**Scheme 16**).



Scheme 16 - Synthesis of 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide (**15**). TBTU - 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro borate; TEA - triethylamine; DMF - dimethylformamide; r.t. - room temperature.

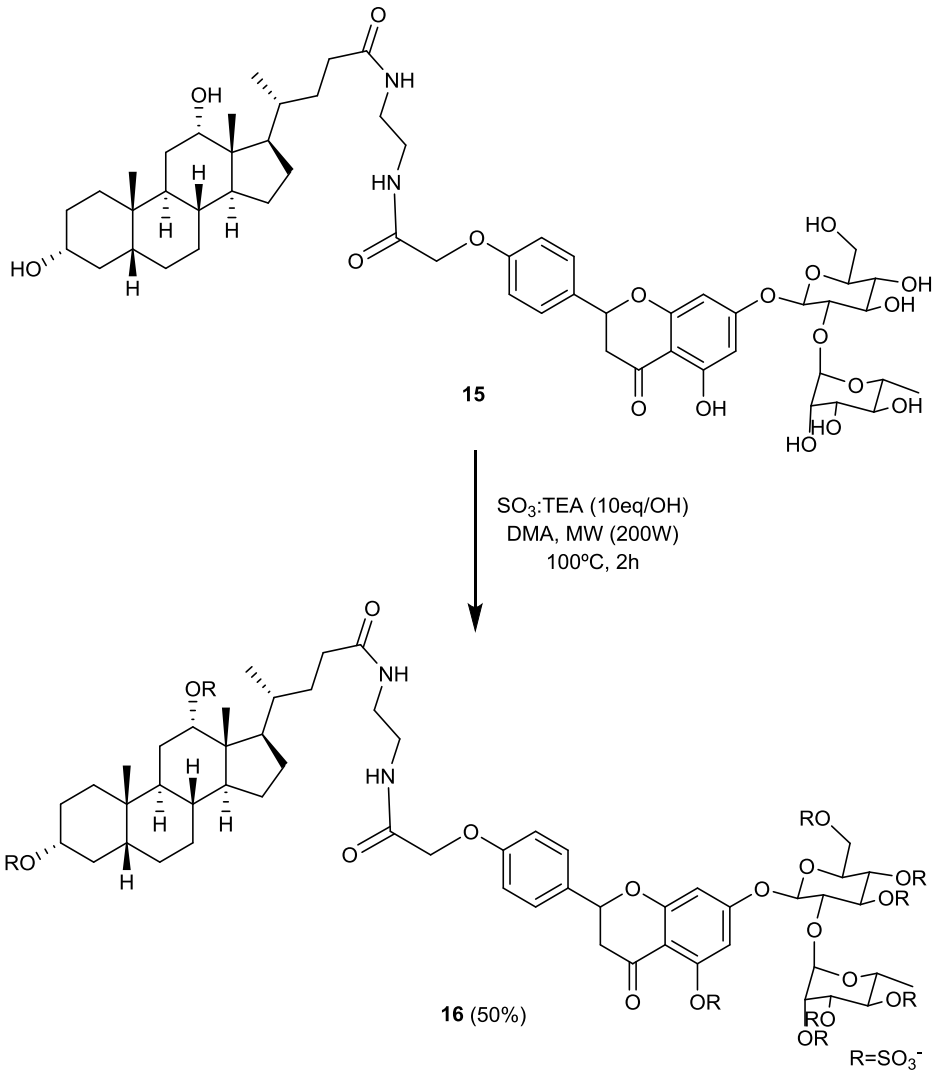
The reaction occurred within 48 hours with 73% yield. After pouring the reaction in ice the product was easily isolated by filtration.

2.1.3.6. Sulfation of 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide (15)

Sulfation of compound **15** was accomplished with MW-assisted sulfation using TEA-sulfur trioxide adduct (SO_3:TEA) in dimethylacetamide (DMA) for 2 hours at 100 ºC in 50% yield (**Scheme 17**).

Sulfur trioxide adducts are successfully applied in the polysulfation and persulfation of polyhydroxyl molecules and are mild reagents when comparing with other sulfation reagents.[120] The use of SO_3:TEA was selected due to successful application in the sulfation of alcohols in carbohydrate scaffolds and sulfation of phenols.[121] MW-assisted sulfation

overcomes some limitations of the conventional methods such as long times of reaction and the use of high amount of sulfation adduct.[122]



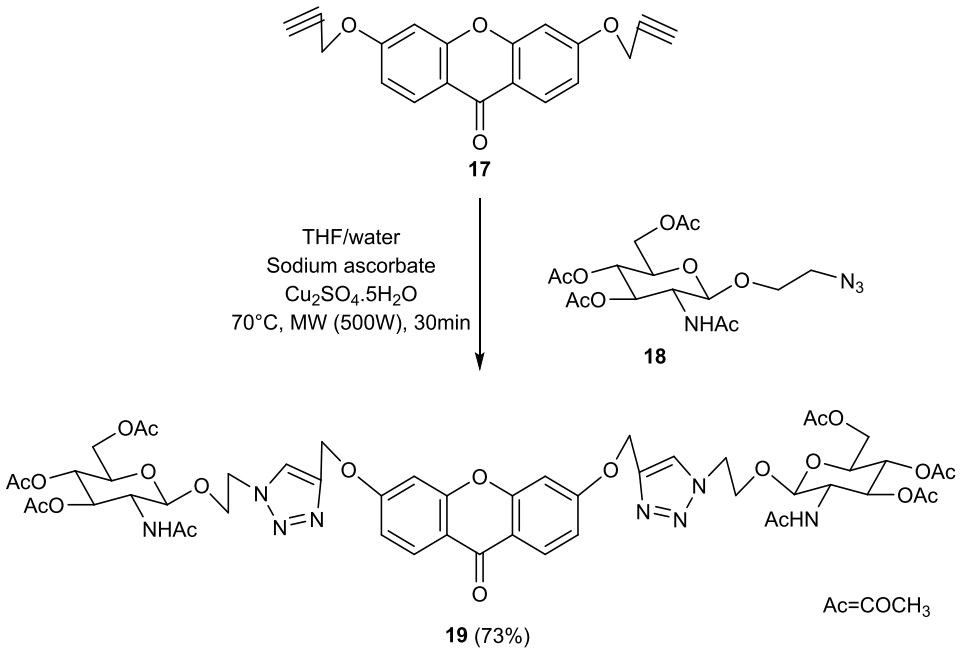
Scheme 17 - Synthesis of 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide persulfate (**16**). SO_3:TEA - triethylamine-sulfur-trioxide adduct; DMA - dimethylacetamide; MW - microwave.

After the completion of the reaction the TEA salt of the sulfated conjugate was converted into the sodium salt with an aqueous solution of NaOAc, followed by insolubilization in ethanol (EtOH) to isolate the desirable product **16** in 50% yield.

2.1.4. Glycosylation of 3,6-dihydroxy xanthone through a triazole

2.1.4.1. Copper(I)-catalyzed alkyne-azide cycloaddition

Among the methods available to form a triazole ring, copper(I)-catalyzed alkyne-azide 1,4-cycloaddition was selected due to several advantages.[123] 3,6-bis(1-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**19**) was synthesized reacting 3,6-bis(prop-2-yn-yloxy)-*9H*-xanthen-9-one (**17**, previously synthesized in LQOF) with 2-azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside (**18**) (**Scheme 18**). After 30 minutes at 70ºC under MW irradiation, compound **19** was easily purified by liquid-liquid extraction and obtained in 73% yield.

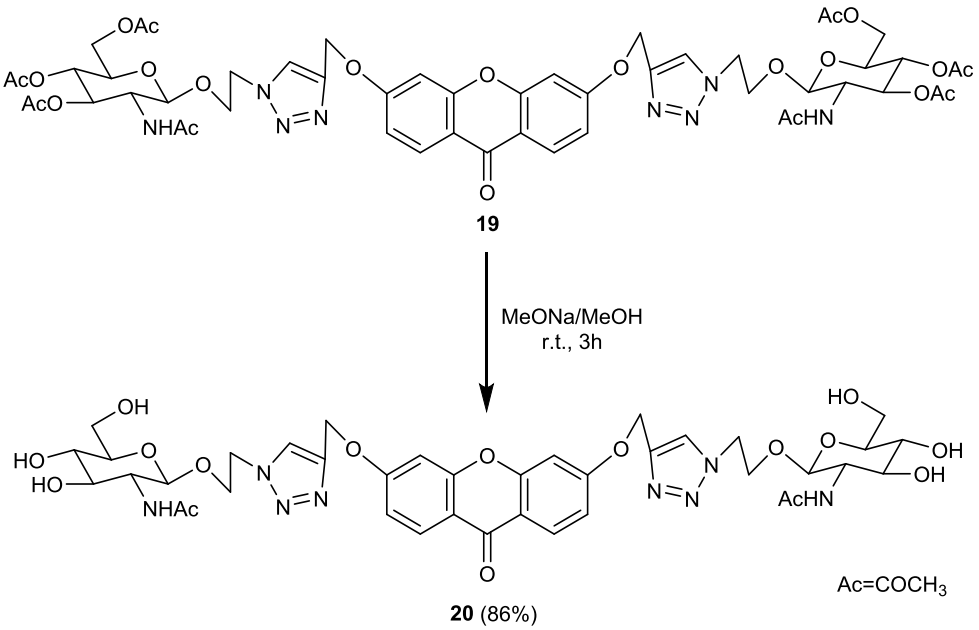


Scheme 18 - Synthesis of 3,6-bis(1-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**19**). THF – tetrahydrofuran; MW - microwave.

2.1.4.2. Deacetylation of 3,6-bis(1-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (19)

Deprotection of the glycosidic moiety of compound **19** was achieved under Zemplén conditions[123] (**Scheme 19**) in 86% yield. The reaction was complete after 3 hours and 3,6-bis(1-(2-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**20**) was easily isolated through filtration. Spectroscopic data

revealed that the acetyl group of the amide was not removed using these reaction conditions.



Scheme 19 - Synthesis of 3,6-bis(1-(2-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**20**). MeONa - sodium methoxide; MeOH - methanol; r.t. - room temperature.

2.1.4.3. *N*-deacetylation of 3,6-bis(1-(2-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (20)

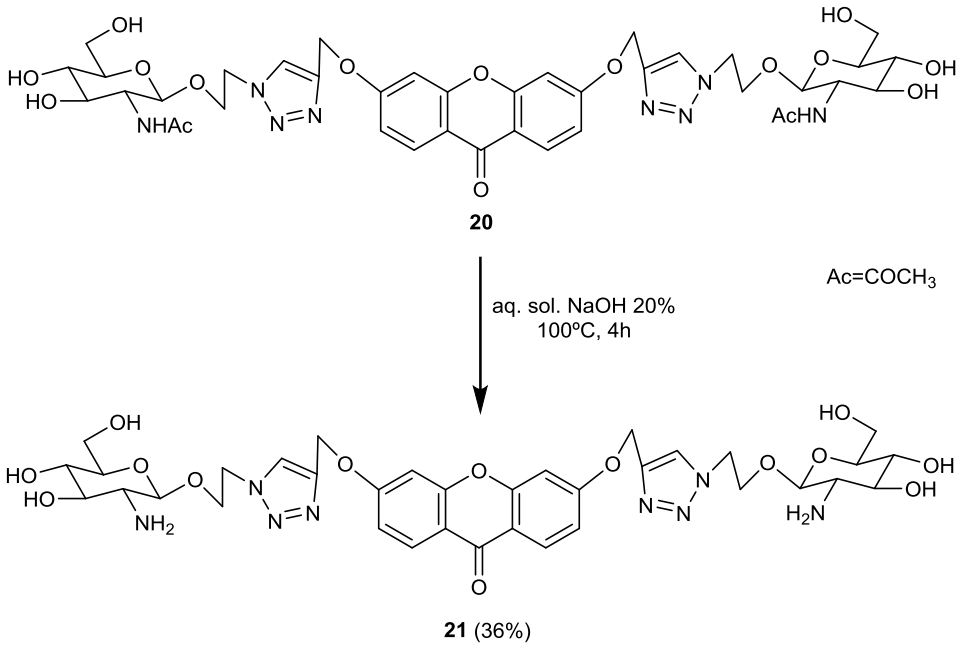
Hydrolysis of amide bonds is achieved generally using harsh conditions, that is, strong acid or base at high temperatures, which could affect other sensitive groups in the molecule.[124] The presence of electronegative groups in carbon or nitrogen accelerates basic catalysis, while alkyl groups in nitrogen retard both basic and acid catalysis.[125]

Some synthetic conditions were attempt for deacetylation of *O*- and *N*-acetyl groups of compound **20**. Firstly, a recent method that use mild conditions - Schwartz' reagent (bis(cyclopentadienyl)zirconium(IV) chloride hydride) - was applied, as an alternative to acid or base-catalysed hydrolysis.[126, 127] However, during the reaction the starting material **20** did not react probably because of low solubility.

A MW-assisted deacetylation method using ammonium salt and EDA was also attempt.[127] Although this method was successfully applied to a wide range of amides, a complex mixture of compounds was obtained.

N-deacetylation was only achieved using a classic base-catalysed hydrolysis, with an aqueous solution of NaOH 20% at 100ºC (**Scheme 20**) after hydrolysis of the *O*-acetyl

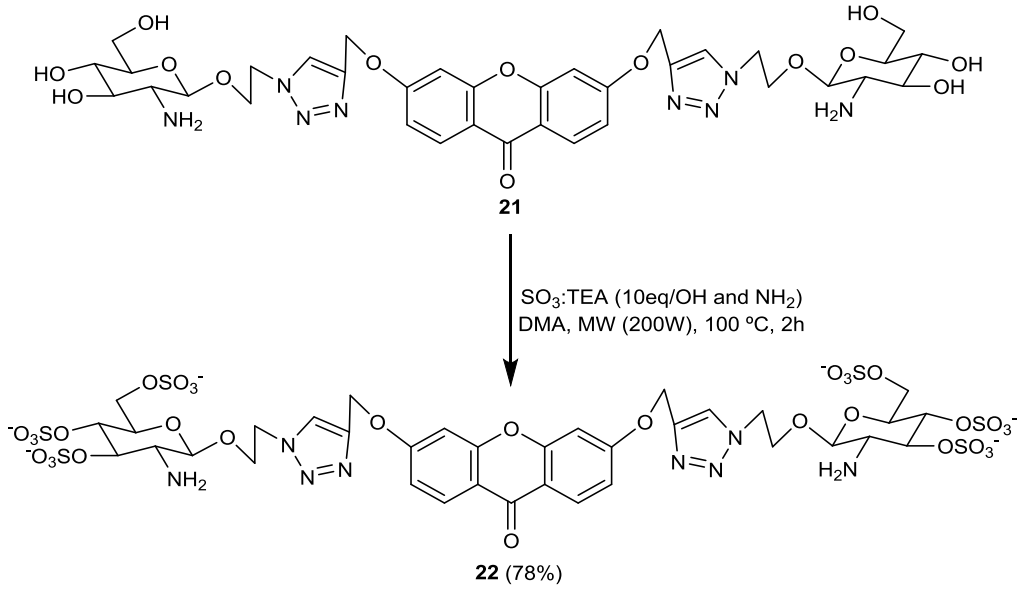
groups using sodium methoxide. Purification was carried out through dialysis following by filtration to furnish compound **21** in 36% yield.



Scheme 20 - Synthesis of 3,6-bis(1-(2-(2-amino-3,4,6-hydroxy-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**21**).

2.1.4.4. Sulfation of 3,6-bis(1-(2-(2-amino-3,4,6-hydroxy-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (21)

Sulfation of compound **21** was achieved under MW irradiation with SO_3:TEA in 2 hours with 78% yield after isolation by a similar procedure as compound **16** (**Scheme 21**).



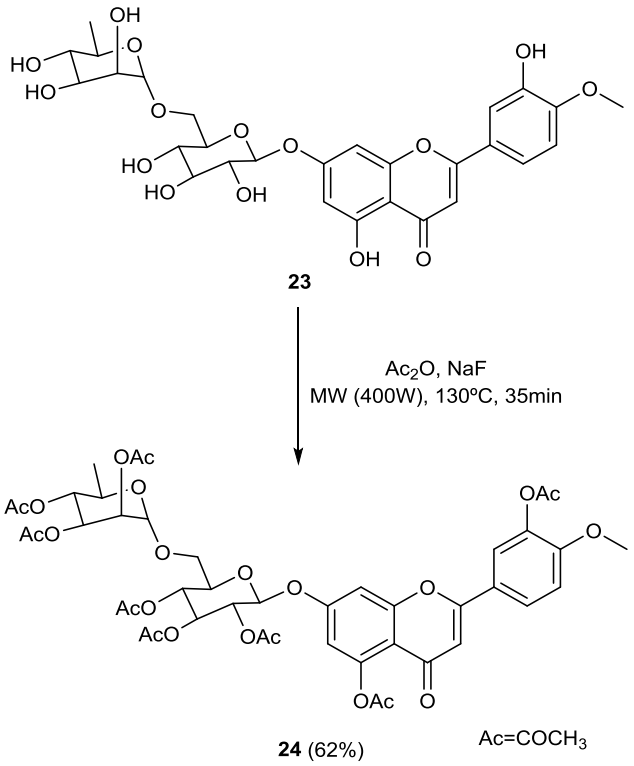
Scheme 21 - Synthesis of 3,6-bis(1-(2-(2-amino-3,4,6-tri-*O*-sulfate-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**22**). SO_3:TEA - triethylamine-sulfur trioxide adduct; DMA - dimethylacetamide; MW - microwave.

2.1.5. Others

In the course of this dissertation, to establish future structure-activity relationships with the final sulfated products for anticoagulant activity or with acetylated intermediates for antitumor activity other phenolic derivatives were obtained and characterized.

2.1.5.1. Acetylation of diosmin (23)

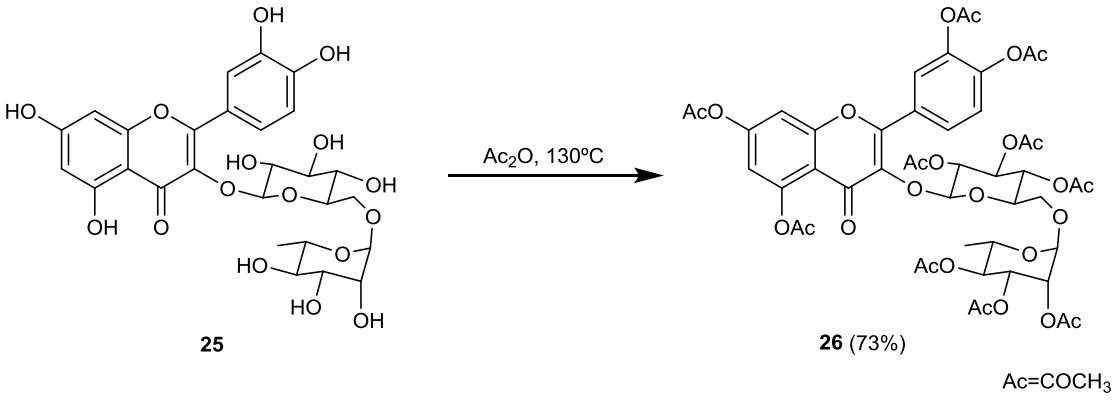
Diosmin (**23**) was acetylated under MW irradiation using NaF as catalyst to give diosmin peracetate (**24**) (**Scheme 22**). In contrast to mangiferin (**5**), acetylation of compound **23** using these reaction conditions lead to the formation of one major product which was isolated through crystallization from MeOH/H_2O in 63% yield.



Scheme 22 - Synthesis of diosmin peracetate (**24**). Ac_2O - acetic anhydride; MW - microwave.

2.1.5.2. Acetylation of rutin (25)

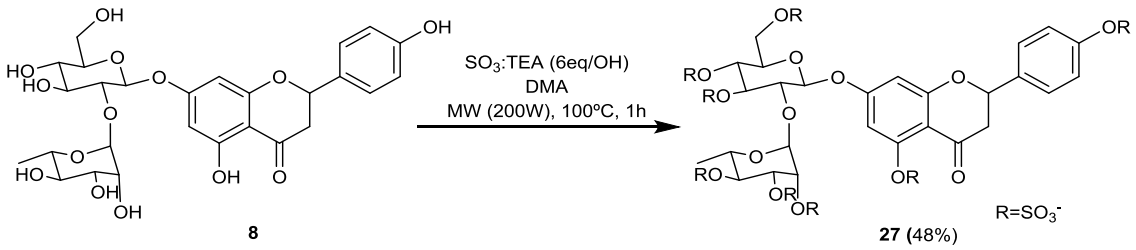
Rutin peracetate (**26**) was obtained through the reaction of rutin (**25**) with acetic anhydride under conventional heating, at 130ºC, with 73% yield (**Scheme 23**). After completion of the reaction the product was isolated through liquid-liquid extraction following by insolubilization.



Scheme 23 - Synthesis of rutin peracetate (**26**). Ac_2O - acetic anhydride.

2.1.5.3. Sulfation of naringin (8)

Naringin persulfate (**27**) was obtained through the reaction of compound **8** with SO_3:TEA, under MW irradiation for 1 hour (**Scheme 24**).



8 **27** (48%)

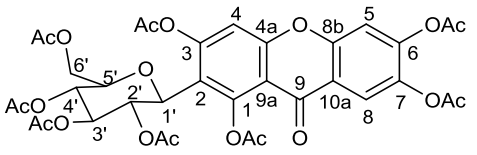
Scheme 24 - Synthesis of naringin persulfate (**27**). SO_3:TEA - triethylamine-sulfur trioxide adduct; DMA - dimethylacetamide; MW - microwave.

Compound **27** was obtained in 48% yield after purification from inorganic salts through dialysis.

2.2. STRUCTURE ELUCIDATION

2.2.1. Mangiferin peracetate (6)

Structure elucidation of mangiferin peracetate (**6**, **Figure 15**) was established by infrared (IR) and nuclear magnetic resonance (NMR) (^{1}H and ^{13}C) and is in accordance with those reported.[99, 128]



6

Figure 15 – Mangiferin peracetate (**6**).

IR spectrum showed two strong bands at 1754 and 1781 cm^{-1} typical of C=O ester stretching vibration which suggested the presence of acetyl groups (**Table 3**).

Table 3 – IR data of compound **6**.

Groups	**υ (cm^{-1})**
	6
C-H aliphatic	2937
C=O ester	1781, 1754
C=O ketone	1664
C=C aromatic	1618, 1459
C-O	1172

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **6** are presented in **APPENDIX I**.

Characteristic signals of the aromatic protons H-4, H-5, and H-8 appeared as singlet at δ_H 7.25, 7.39, and 8.00 ppm, respectively, whereas the corresponding signals of these protons appeared at δ_H 6.38, 6.87, and 7.38 ppm in the non-acetylated parent compound, mangiferin (**5**, also in **APPENDIX I**). Seven signals characteristic of aliphatic protons (the sugar moiety) were observed between δ_H 3.80-5.73 ppm, whereas the corresponding

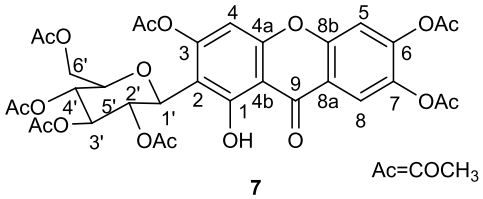
signals of these protons appeared between δ_H 3.09-4.59 ppm in the non-acetylated parent compound, mangiferin (**5**). Eight singlets between δ_H 1.80-2.53 ppm integrating each for three protons were assigned for -CH_3 protons of acetyl groups, which indicated the presence of the peracetylated derivative **6**.

^{13}C NMR spectrum of compound **6** showed eight signals between δ_C 167.2-170.6 ppm, and eight signals between δ_C 20.3-21.4 ppm, which were assigned to eight C=O of the acetyl groups and to eight -CH_3 groups, respectively.

In ^{13}C NMR spectrum of non-acetylated mangiferin (**5**), carbons C-1, 3, 6, and 7 were assigned to δ_C 161.8, 163.9, 154.1, and 143.8 ppm, respectively, whereas in compound **6** these carbons were shielded (δ_C 152.9, 154.4, 152.9, and 139.4 ppm). In contrast, carbons C-4 and C-5, in non-acetylated mangiferin (**5**), were respectively assigned to δ_C 93.3 and 102.7 ppm, whereas in compound **6** these carbons were deshielded (δ_C 111.7 and 112.7 ppm).

2.2.2. Mangiferin heptaacetate (7)

Structure elucidation of mangiferin heptaacetate (**7**, **Figure 16**) was established by IR and NMR (^1H and ^{13}C) and is in accordance with those reported.[99]



7

Figure 16 – Mangiferin heptaacetate (**7**).

The presence of acetyl groups was suggested by the observation of two strong bands at 1779 and 1746 cm^{-1} typical of C=O ester stretch. Additionally, a large band of stretching vibration of the O-H bond at 3443 cm^{-1} suggested at least one free hydroxyl (**Table 4**).

Table 4 – IR data of compound **7**.

Groups	**υ (cm^{-1})**
	7
O-H	3443
C-H aliphatic	2919, 2850
C=O ester	1779, 1746
C=O ketone	1647
C=C aromatic	1619, 1467
C-O	1229

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **7** are also presented in **APPENDIX I**.

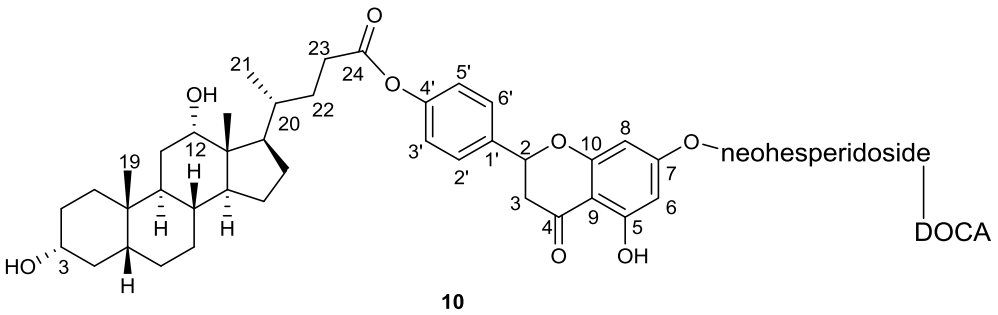
Characteristic signals of the aromatic protons H-4, H-5, and H-8 appeared as singlets at δ_H 6.76, 7.42, and 8.06 ppm, respectively, whereas the corresponding signals of these protons appeared at δ_H 6.38, 6.87, and 7.38 ppm in the non-acetylated parent compound, mangiferin (**5**, also in **APPENDIX I**). Seven signals characteristic of aliphatic protons (the sugar moiety) were observed between δ_H 3.81-5.68 ppm, whereas the corresponding signals of these protons appeared between δ_H 3.09-4.59 ppm in the non-acetylated parent compound, mangiferin (**5**). Seven singlets between δ_H 1.78-2.44 ppm integrating each for three protons were assigned for $-CH_3$ protons of acetyl groups, which indicated the presence of the peracetylated derivative **7**.

^{13}C NMR spectrum showed seven signals between δ_C 167.1-170.5 ppm, and seven signals between δ_C 20.4-22.7 ppm, which were assigned to seven C=O of the acetyl groups and to seven $-CH_3$ groups, respectively.

In ^{13}C NMR spectrum of non-acetylated mangiferin (**5**), carbons C-3, 6, and 7 were assigned to δ_C 163.9, 154.1, and 143.8 ppm, respectively, whereas these carbons were shielded (δ_C 153.9, 148.4, and 139.4 ppm) in compound **7**. In contrast, carbons C-4 and C-5, in non-acetylated mangiferin (**5**), were respectively assigned to δ_C 93.3 and 102.7 ppm, whereas these carbons were deshielded (δ_C 110.6 and 112.9 ppm) in compound **7**.

2.2.3. Naringin-di-deoxycholate (10)

Structure elucidation of naringin-di-deoxycholate (**10**, **Figure 17**) was established by IR, NMR (^{1}H and ^{13}C), HSQC, and HMBC.



10

Figure 17 – Naringin-di-deoxycholate (**10**).

IR spectrum showed a band typical of C=O ester stretch at 1737 cm^{-1} (**Table 5**).

Table 5 – IR data of compound **10**.

Groups	**υ cm^{-1}**
	10
O-H	3415
C-H aliphatic	2933, 2865
C=O ester	1737
C=O ketone	1642
C-O	1087

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **10** are presented in **APPENDIX IV**.

^{1}H NMR spectrum showed signals of the flavanone glycoside position and of the steroid scaffold. The number of protons indicated the presence of two molecules of compound **9**. Typical signals of the aromatic protons H-2', H-6' and H-3', H-5' appeared as duplet at δ_H 7.56 and 7.17 ppm, respectively, whereas the corresponding signals of these protons appeared at δ_H 7.33 and 6.80 ppm in naringin (**8**) indicating that molecular modification occurred at 4'-position. Additionally, ^{1}H NMR spectrum showed a singlet at δ_H 12.03 ppm that was assigned for 5-OH.

^{13}C NMR spectra showed two signals at δ_C 173.2 and 172.2 ppm that indicate the presence of two carbons typical of C=O ester groups. Carbon C-4' was assigned to δ_C 150.7 ppm, whereas the same carbon was assigned to δ_C 157.9 ppm in compound **8**.

The assignments of carbon atoms directly bonded to proton atoms were achieved from HSQC experiments and the chemical shifts of the carbon atoms not directly bonded to proton atoms were deduced from HMBC correlations (**Figure 18**).

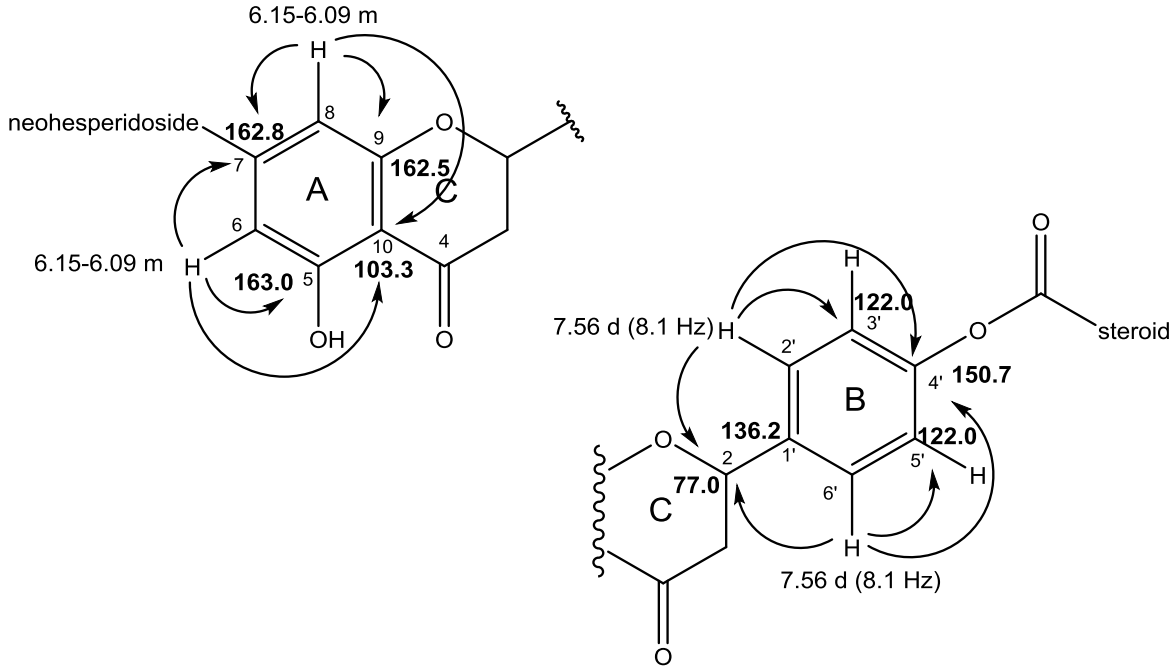


Figure 18 – Main connectivities found in HMBC for compound **10**.

2.2.4. Methyl 4'-naringin acetate (11)

Structure elucidation of methyl 4'-naringin acetate (**11**, **Figure 19**) was established for the first time by IR, NMR (^1H and ^{13}C), HSQC, and HMBC.

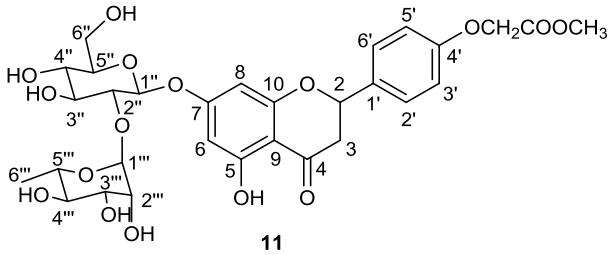


Figure 19 – Methyl 4'-naringin acetate (**11**).

IR spectrum of compound **11** showed a band at 1754 cm^{-1} characteristic of a C=O ester stretch which is in accordance with the molecular modification performed (**Table 6**).

Table 6 – IR data of compound **11**.

Groups	**υ cm^{-1}**
	11
O-H	3420
C-H aliphatic	2922
C=O ester	1754
C=O ketone	1632
C=C aromatic	1511

υ - wavenumber

^{1}H and ^{13}C NMR data of compound **11** is presented in **APPENDIX II**.

^{1}H and ^{13}C NMR spectra of compound **11** showed signals that indicate the presence of only one carbomethoxymethyl group, namely signals corresponding to the methyl group (δ_H 3.71 and δ_C 51.9 ppm) and to the methylene bridge (δ_H 4.83 and δ_C 65.4 ppm).

Characteristic signals of the aromatic protons H-2', H-5', and H-3', H-6' appeared at δ_H 7.46 and 7.02-6.97 ppm, respectively, whereas the corresponding signals of the same protons appeared at δ_H 7.33 and 6.80 ppm in the parent compound, naringin (**8**, also in **APPENDIX II**). Signals of the hydroxyl protons of the sugar moiety (δ_H 4.83-5.15 ppm) and the hydroxyl proton at C-5 (δ_H 12.05 ppm) were also observed in the ^{1}H NMR spectrum of compound **11**.

The assignments of carbon atoms directly bonded to proton atoms were achieved from HSQC experiments and the chemical shifts of the carbon atoms not directly bonded to proton atoms were deduced from HMBC correlations (**Figure 20**). The position of the methylacetate group on naringin was further confirmed by the correlations observed in HMBC spectrum between the signal of methylene protons (δ_H 4.83 ppm) and the signal of C-4' (δ_C 157.9 ppm) (**Figure 20**).

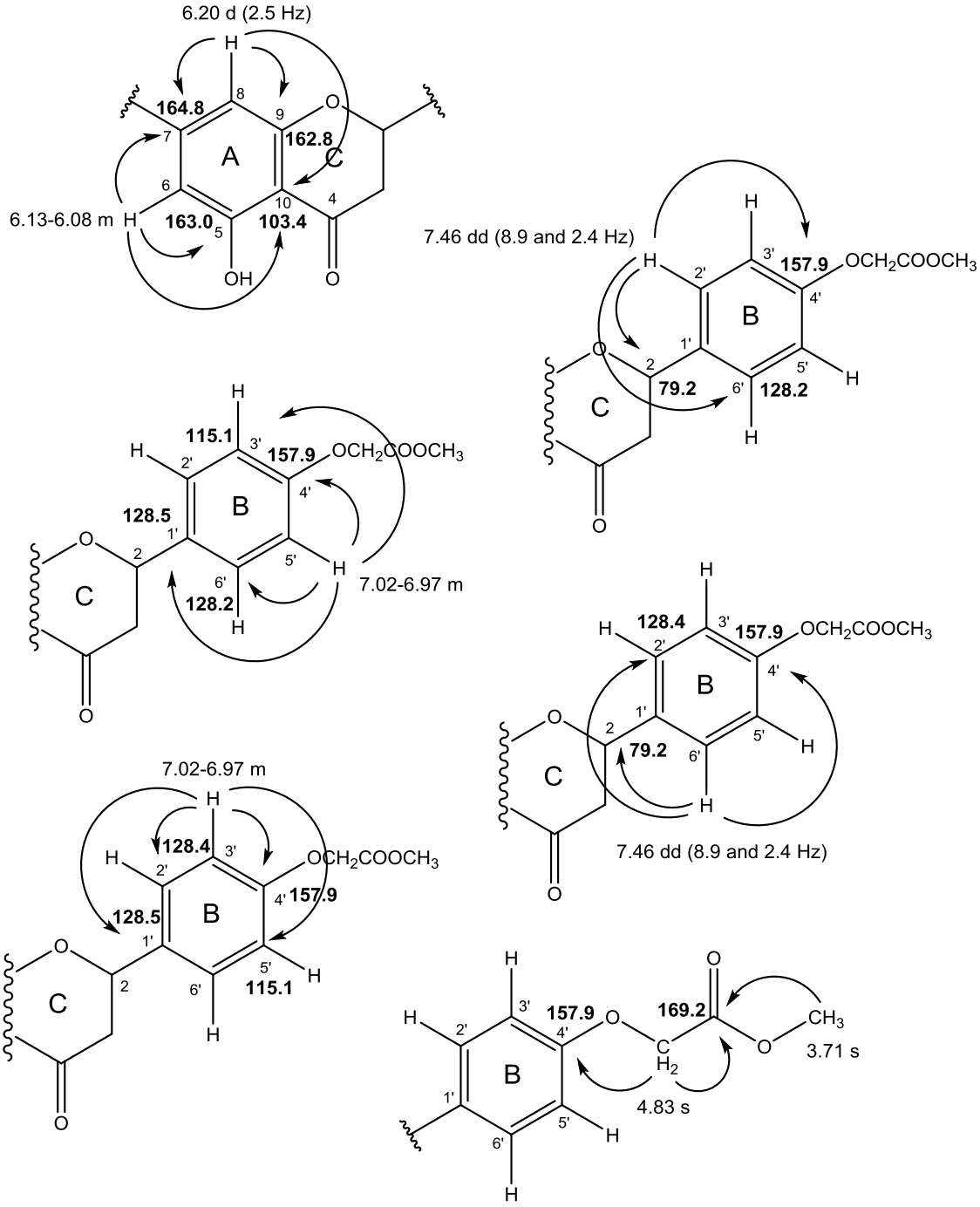


Figure 20 – Main connectivities found in HMBC for compound **11**.

2.2.5. 4'-Naringin acetic acid (12)

Structure elucidation of 4'-naringin acetic acid (**12**, **Figure 21**) was established for the first time by IR and NMR (^{1}H and ^{13}C).

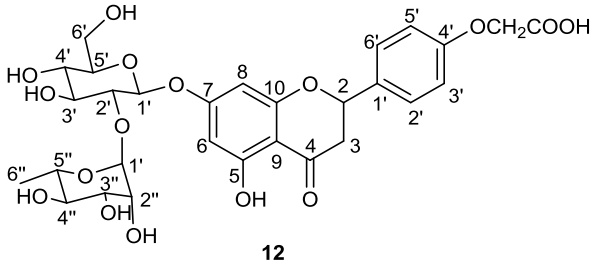


Figure 21 – 4'-Naringin acetic acid (**12**).

IR spectrum of compound **12** showed a band at 1735 cm^{-1} from the C=O carboxylic acid stretch suggesting the presence of the carboxylic acid group (**Table 7**).

Table 7 – IR data of compound **12**.

Groups	**υ (cm^{-1})**
	12
O-H	3438
C-H aliphatic	2919
C=O carboxylic acid	1735
C=O ketone	1628
C=C aromatic	1512

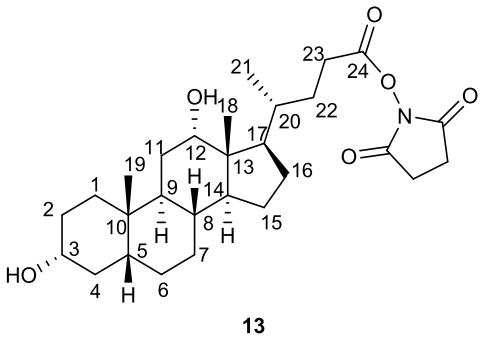
υ - wavenumber

^{1}H and ^{13}C NMR data for compound **12** are also presented in **APPENDIX II**.

^{1}H NMR spectrum showed a signal at δ_H 13.89 ppm indicating the presence of an acidic proton in the carboxylic acid group. When comparing with methyl 4'-naringin acetate (**11**), the ^{1}H NMR and ^{13}C NMR spectra of compound **12** did not show a singlet signal around δ_H 3.71 ppm neither a signal corresponding to a $-CH_3$ carbon of an acetate group.

2.2.6. Succinimido deoxycholate (13)

Structure elucidation of DOCA-NHS (**13**, **Figure 22**) was established by IR and NMR (^{1}H and ^{13}C). The ^{1}H NMR is in accordance with those reported.[40]



13

Figure 22 – Succinimido deoxycholate (DOCA-NHS, **13**).

IR spectrum of compound **13** showed bands at 1811, 1781, and 1741 cm^{-1} typical of the C=O stretch vibration (**Table 8**).

Table 8 – IR data of compound **13**.

Groups	**υ (cm^{-1})**
	13
O-H	3432
C-H aliphatic	2938, 2863
C=O (NHS)	1811, 1781
C=O ester	1741

υ - wavenumber

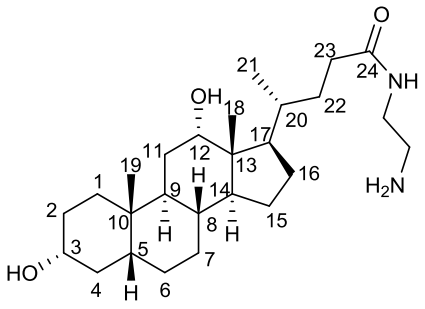
^{1}H and ^{13}C NMR data for compound **13** are presented in **APPENDIX III**.

^{1}H NMR spectrum showed a multiplet signal integrating for four protons between δ_H 2.82-2.87 ppm that was assigned for the aliphatic protons of NHS.

^{13}C NMR spectrum showed a signal at δ_C 169.2 ppm that was assigned to the two C=O of NHS. Additionally, when comparing with DOCA (**9**), ^{1}H NMR spectra of compound **13** did not show a singlet signal of the acidic proton between δ_H 12-13 ppm.

2.2.7. *N*-Deoxycholylethylenediamine (14)

Structure elucidation of DOCA-NH_2 (**14**, **Figure 23**) was established for the first time by IR and NMR (^{1}H and ^{13}C). Although compound **14** is already described in the literature, this is the first complete characterization of compound **14**.



14

Figure 23 – *N*-Deoxycholylethylenediamine (DOCA-NH_2, **14**).

The IR spectrum showed two bands at 1558 and 1628 cm^{-1} of the N-H bend, and a band at 671 cm^{-1} of the N-H wag (**Table 9**). Overlapping occured in the observed position of N-H and O-H stretching frequencies so the presence of a primary amine is difficult to confirm.

Table 9 – IR data of compound **14**.

Groups	**υ (cm^{-1})**
	14
O-H/N-H stretch	3600-3200
C-H aliphatic	2926, 2861
C=O amide	1694
N-H bend	1628, 1558
N-H wag	671

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **14** are presented in **APPENDIX III**.

^{1}H NMR spectrum showed a triplet integrating for one proton at δ_H 7.72 ppm typical of a secondary amine proton and a quartet integrating for four protons at δ_H 3.01 ppm that was assigned to the methylene protons of the EDA aliphatic chain.

^{13}C NMR spectrum showed two signals from the carbons of the methylene groups of the EDA aliphatic chain (δ_C 41.4 and 41.6 ppm).

2.2.8. 4'-Naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide (15)

Structure elucidation of 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide (**15**, **Figure 24**) was established for the first time by IR and NMR (^{1}H and ^{13}C).

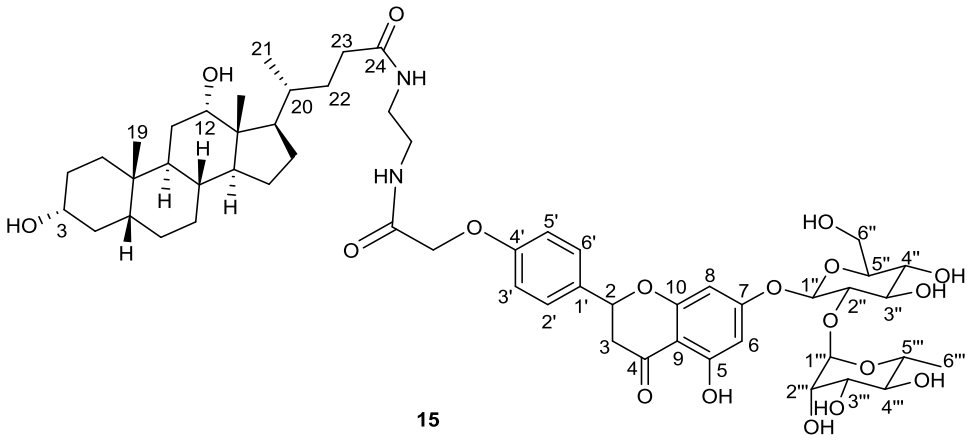


Figure 24 – 4'-Naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide (**15**).

IR spectrum showed bands of the O-H stretch at 3443 cm^{-1} (**Table 10**). The band of the N-H amide, the C=O ketone stretch, and the C=O amide stretch were overlapped.

Table 10 – IR data of compound **15**.

Groups	**υ (cm^{-1})**
	15
O-H	3443
C-H aliphatic	2924
C=O ketone/amide/N-H	around 1633

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **15** are presented in **APPENDIX IV**.

^{1}H NMR spectrum showed singlet signals of two protons of the amide bonds between δ_H 7.79-7.87 ppm and at δ_H 8.17 ppm which indicate the success of the coupling reaction between compound **12** and compound **14**.

^{13}C NMR spectrum showed two signals at at δ_C 174.3 and 168.6 ppm corresponding to the C=O of amide bonds.

2.2.9. 4'-Naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide persulfate (16)

Structure elucidation of 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide persulfate (**16**, **Figure 25**) was established for the first time by IR, NMR (^{1}H and ^{13}C), HSQC, and HMBC.

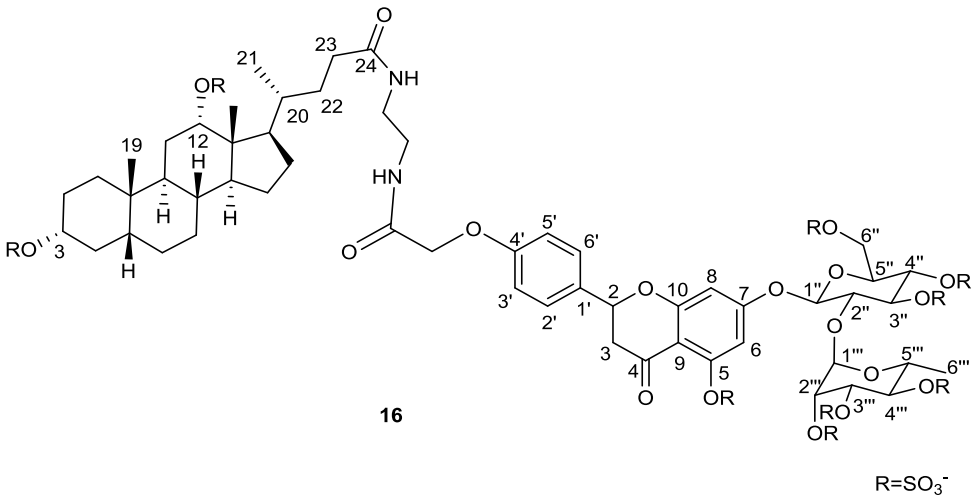


Figure 25 – 4'-Naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide persulfate (**16**).

IR spectrum showed bands from the sulfate groups at 1240 cm^{-1} (S=O), 1058 cm^{-1} (C-O-S), and 810 cm^{-1} (S-O) (**Table 11**).

Table 11 – IR of compound **16**.

Groups	**υ (cm^{-1})**
	16
C-H aliphatic	2921, 2863
C=O ketone/amide	1632
N-H bend	1558
S=O	1240
C-O-S	1058
S-O	810

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **16** is presented in **APPENDIX IV**.

^{1}H NMR spectrum of compound **16** did not show signals in the typical region of hydroxyl proton signals (δ_H 4.53-5.36 ppm) when compared to compound **15**. Twelve signals characteristic of aliphatic protons (the sugar moiety) were observed between δ_H 3.70-4.75 ppm in compound **16**, whereas the corresponding signals of these protons appeared between δ_H 3.35-3.77 ppm in the non-sulfated parent compound, 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide (**15**, also in **APPENDIX IV**). Signals of H-3 and H-12 of the steroid moiety appeared at δ_H 3.70-4.01 and 4.42-4.53 ppm, respectively, whereas the corresponding signals of these protons appeared at δ_H 3.70-4.01 and 4.42-4.53 ppm in the non-sulfated parent compound, 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide (**15**).

^{13}C NMR spectrum showed signals of the C=O group of the amide bond at δ_C 173.1 and 167.8 ppm. Signals of the ethyl linker between the amide bonds were observed at δ_C 38.4 and 38.2 ppm, and of the 4'-OCH_2 at δ_C 67.0 ppm.

The assignments of the carbon atoms directly bonded to proton atoms were achieved from HSQC experiments and the chemical shifts of the carbon atoms not directly bonded to proton atoms were deduced from HMBC correlations (**Figure 26**).

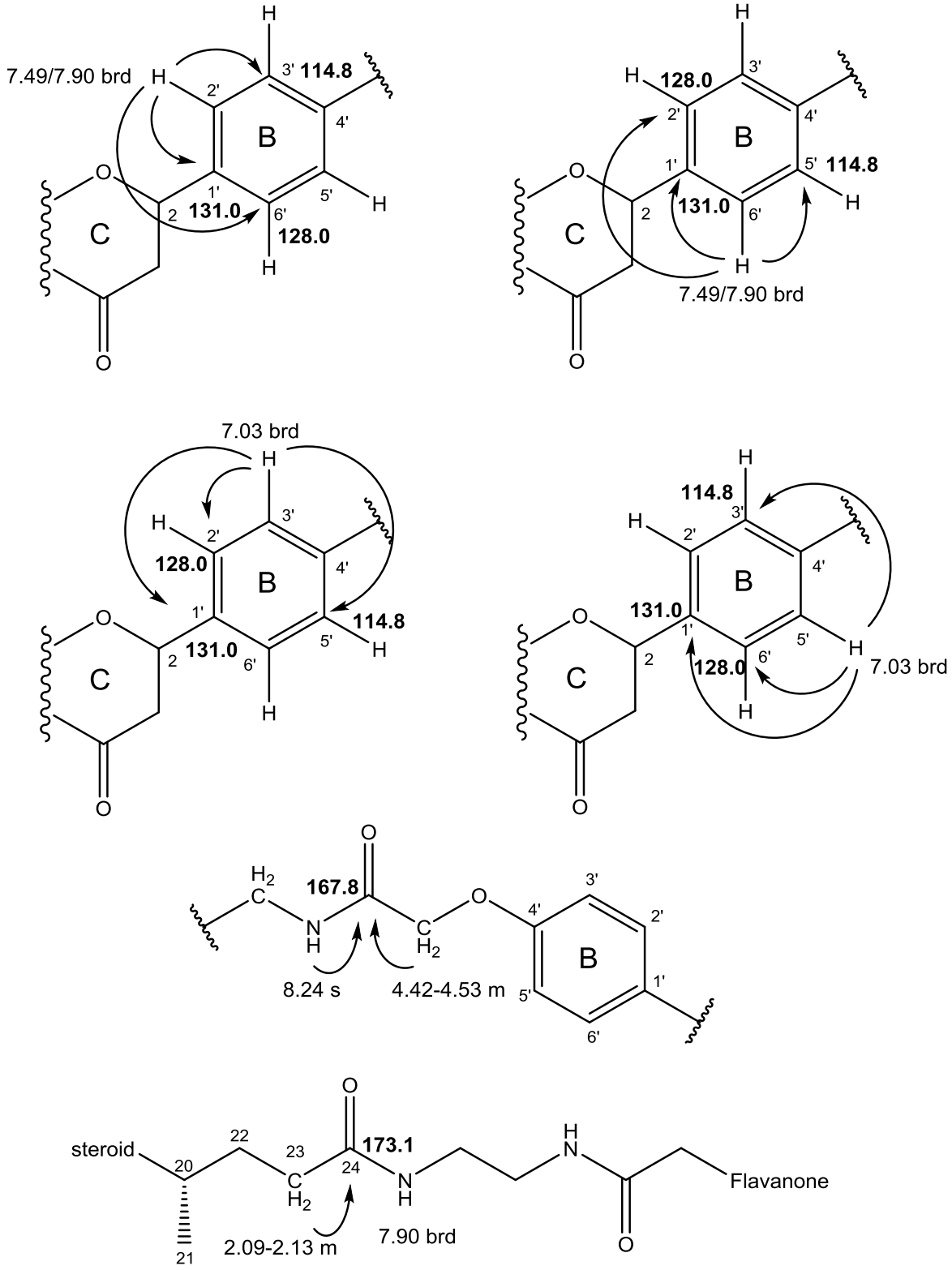
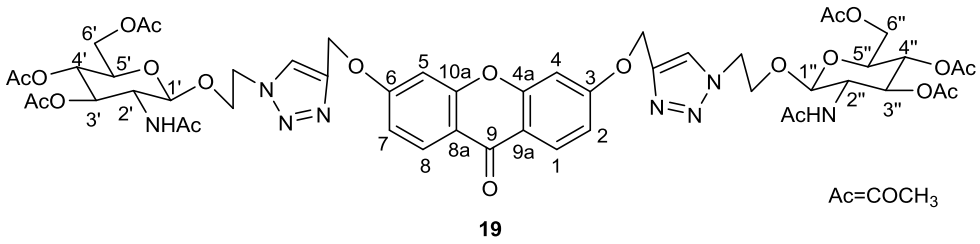


Figure 26 - Main connectivities found in HMBC for compound **16.**

2.2.10. 3,6-Bis(1-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (19)

Structure elucidation of 3,6-bis(1-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**19**, **Figure 27**) was established for the first time by IR, NMR (^{1}H and ^{13}C), HSQC and HMBC.



19

Figure 27 – 3,6-bis(1-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**19**).

IR spectrum showed one band at 1745 cm^{-1} of C=O ester stretch (**Table 12**) and also a band at 2957 cm^{-1} of C-H stretch that suggest the presence of a sugar moiety.

Table 12 – IR data of compound **19**.

Groups	**υ (cm^{-1})**
	19
C-H aliphatic	2957
C=O ester	1745
C=O ketone/amide	1660
N-H bend	1609

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **19** are presented in **APPENDIX V**.

The success of the molecular modification performed was confirmed by ^{1}H NMR and ^{13}C NMR that showed signals of the triazole ring (δ_C 125.4 ppm, δ_H 8.15 ppm, and δ_C 141.5 ppm of CH=C, respectively) and of the sugar moiety (δ_H 1.71-2.02 ppm and δ_C 20.2-22.6 ppm of the -CH_3 groups, and δ_C 169.1-170.1 ppm of the C=O of the acetyl groups).

Assignments of the carbon atoms directly bonded to proton atoms were achieved from HSQC experiments and the chemical shifts of the carbon atoms not directly bonded to proton atoms were deduced from HMBC correlations (**Figure 28**).

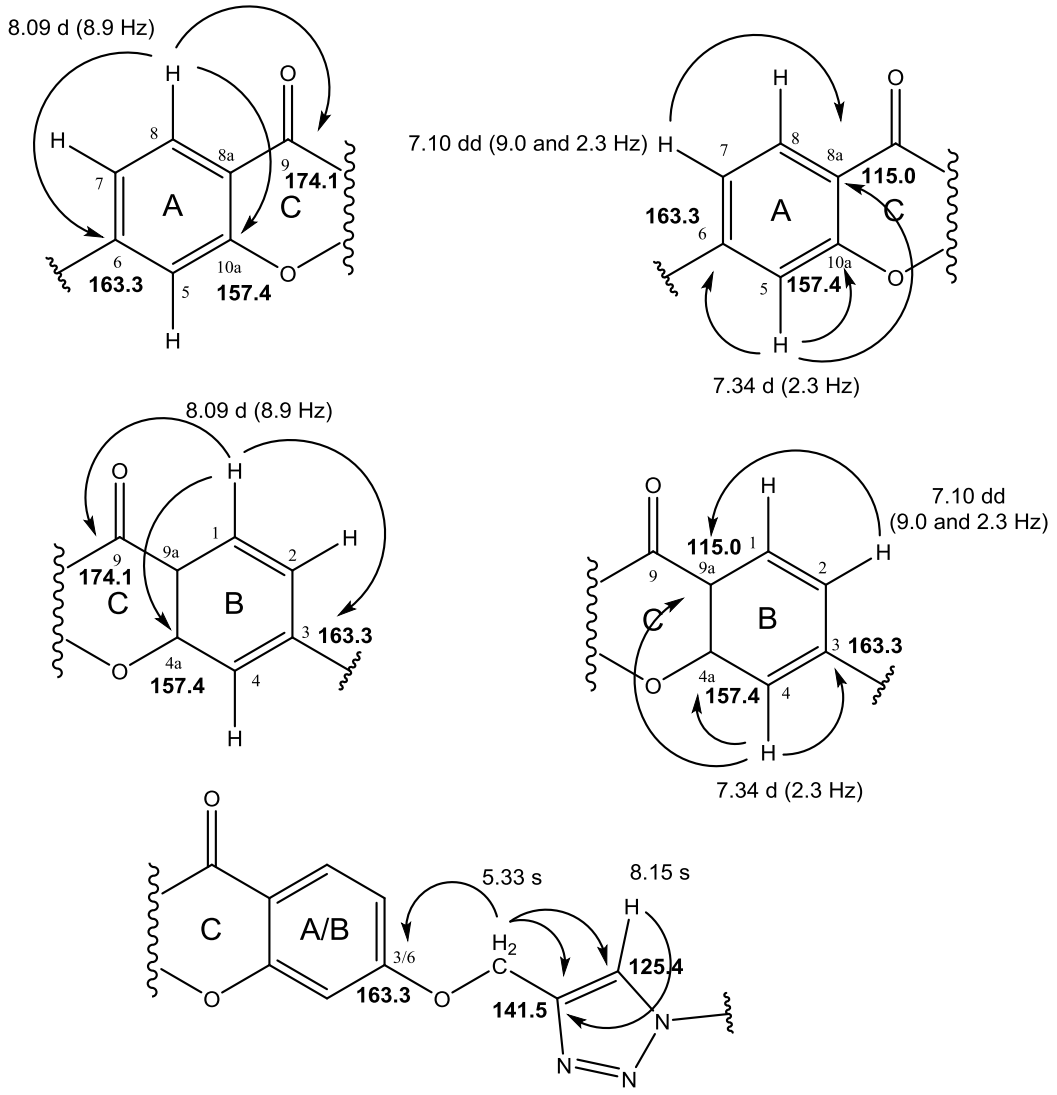
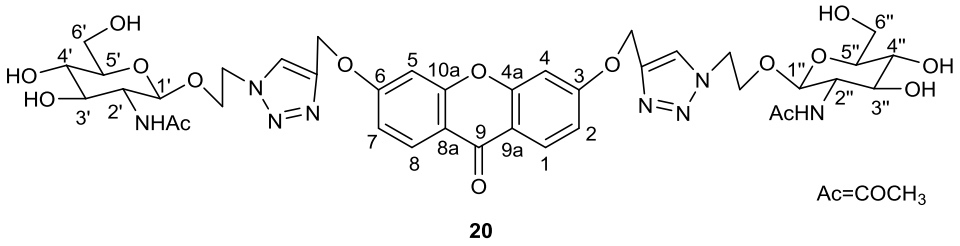


Figure 28 – Main connectivities found in HMBC for compound **19**.

2.2.11. 3,6-Bis(1-(2-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (20)

Structure elucidation of 3,6-bis(1-(2-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**20**, **Figure 29**) was established for the first time by IR, NMR (^{1}H and ^{13}C), HSQC and HMBC.



20

Figure 29 - 3,6-Bis(1-(2-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**20**).

IR spectrum of compound **20** did only present a band at 1648 cm^{-1} typical of the C=O ketone stretch which suggest that acetyl groups were removed (**Table 13**).

Table 13 – IR data of compound **20**.

Groups	**υ (cm^{-1})**
	20
O-H	3415
C-H aliphatic	2919
C=O ketone/amide	1648
N-H bend	1610

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **20** are presented in **APPENDIX V**.

^{1}H NMR spectrum showed signals between δ_H 4.57-5.08 ppm which is in accordance with the presence of hydroxyl protons.

In ^{13}C NMR spectrum of compound **19**, carbons C-1', C-3', and C-4' were assigned to δ_C 99.8, 68.4, and 70.7 ppm, respectively, whereas these carbons were deshielded (δ_C 100.7, 70.5, and 74.1 ppm) in compound **20**.

The assignments of the carbon atoms directly bonded to proton atoms were achieved from HSQC experiments and the chemical shifts of the carbon atoms not directly bonded to proton atoms were deduced from HMBC correlations (**Figure 30**).

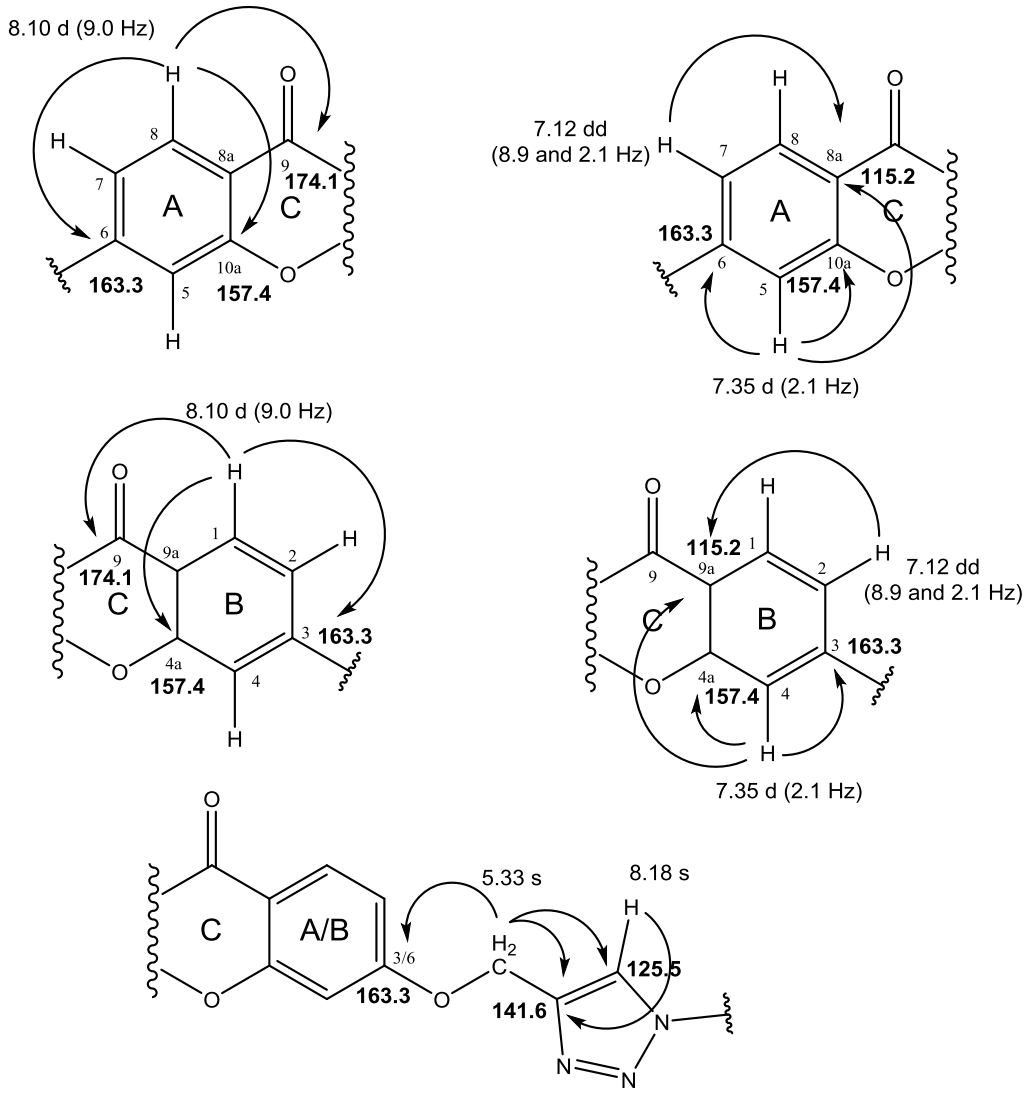
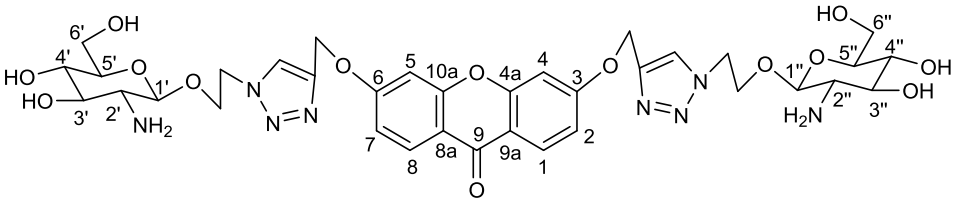


Figure 30 – Main connectivities found in HMBC for compound **20**.

2.2.12. 3,6-Bis(1-(2-(2-amino-3,4,6-hydroxy-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (21)

Structure elucidation of 3,6-bis(1-(2-(2-amino-3,4,6-hydroxy-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**21**, **Figure 31**) was established for the first time by IR, NMR (^{1}H and ^{13}C).



21

Figure 31 – 3,6-Bis(1-(2-(2-amino-3,4,6-hydroxy-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**21**).

The IR spectrum showed bands of N-H bend and of N-H wag at 1609 and 841 cm^{-1}, respectively (**Table 15**). Overlapping occured in the observed position of N-H and O-H stretching frequencies so the presence of a primary amine is difficult to confirm.

Table 14 – IR data of compound **21**.

Groups	**υ (cm^{-1})**
	21
O-H	3362
C-H aliphatic	2920, 2880
C=O ketone/amide	1641
N-H bend	1609
N-H wag	841

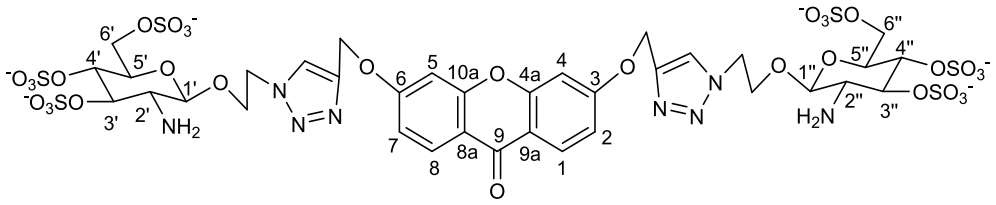
υ - wavenumber

^{1}H and ^{13}C NMR data for compound **21** are presented in **APPENDIX V**.

Comparing with the starting material, compound **20**, the ^{1}H NMR spectrum of compound **21** did not show the signal in the typical region of the $-CH_3$ protons of the acetamide group ($-NHCOCH_3$). A singlet signal of two protons at δ_H 1.44 ppm was observed which indicate the presence of a primary amine.

2.2.13. 3,6-Bis(1-(2-(2-amino-3,4,6-tri-*O*-sulfate-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (22)

Structure elucidation of 3,6-bis(1-(2-(2-amino-3,4,6-tri-*O*-sulfate-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**22**, **Figure 32**) was established for the first time by IR, NMR (^{1}H and ^{13}C), HSQC, and HMBC.



22

Figure 32 – 3,6-Bis(1-(2-(2-amino-3,4,6-tri-*O*-sulfate-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**22**).

IR spectrum showed bands at 1257 cm^{-1} (C-O-S), 1047 cm^{-1} (S=O), and 814 cm^{-1} (S-O) (**Table 15**) which suggests the presence of sulfate groups.

Table 15 - IR data of compound **22**.

Groups	**υ (cm^{-1})**
	22
C-H aliphatic	2917, 2847
C=O ketone/amide	1642
N-H bend	1619
C=O	1257
C-O-S	1047
S-O	814

υ - wavenumber

^{1}H and ^{13}C NMR of compound **22** are presented in **APPENDIX V**.

When comparing with the starting material, compound **21**, signals of hydroxyl protons were not observed in the ^{1}H NMR spectrum of compound **22**. ^{1}H NMR spectrum also showed a signal at δ_H 1.73 ppm integrated for two protons which indicate that the amine group was not modified.

In ^{13}C NMR spectrum of compound **21**, carbons C-1', C-3', C-4', and C-6' were assigned to δ_C 101.5, 70.9, 76.5, and 61.1 ppm, respectively, whereas these carbons were shielded (δ_C 100.5, 70.5, 74.5, and 60.9 ppm), in compound **22.**

The assignments of the carbon atoms directly bonded to proton atoms were achieved from HSQC experiments and the chemical shifts of the carbon atoms not directly bonded to proton atoms were deduced from HMBC correlations (**Figure 33**).

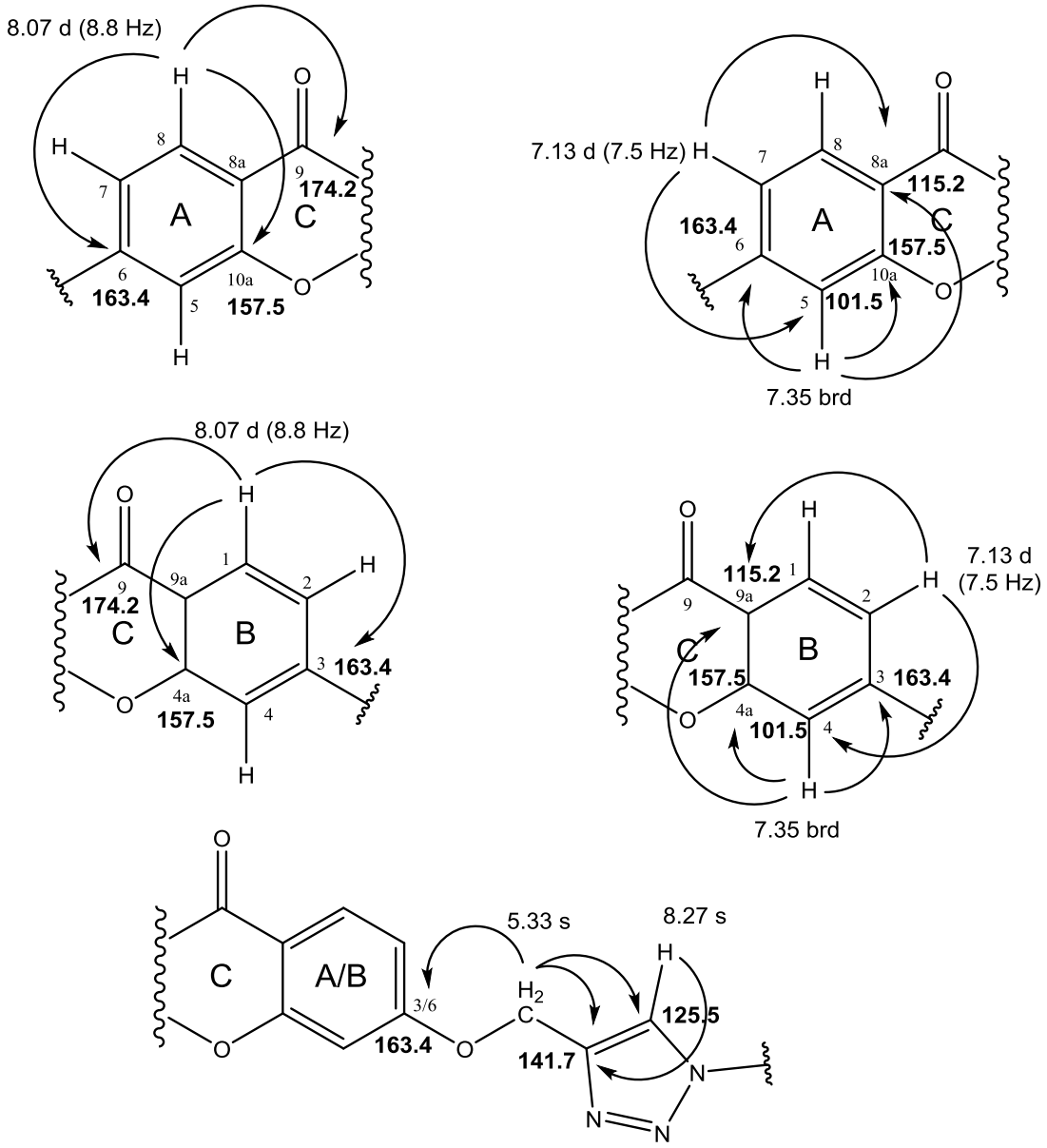


Figure 33 - Main connectivities found in HMBC for compound **22**.

2.2.14. Diosmin peracetate (24)

Structure elucidation of diosmin peracetate (**24**, **Figure 34**) was established by IR and NMR (^{1}H and ^{13}C). The ^{1}H NMR is in accordance with those reported.[129]

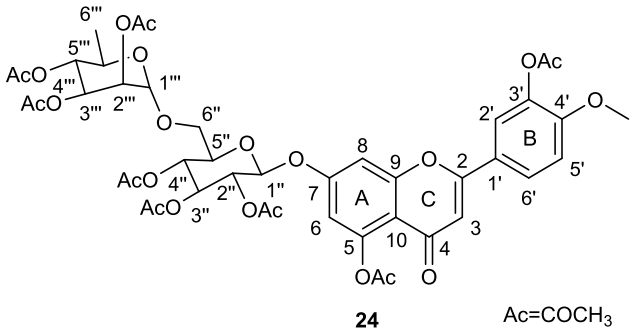


Figure 34 – Diosmin peracetate (**24**).

The IR spectrum showed a band at 1754 cm^{-1} (C=O ester stretch) that suggests the presence of acetyl groups (**Table 16**).

Table 16 – IR data of compound **24**.

Groups	**υ (cm^{-1})**
	24
C-H aliphatic	2940
C=O ester	1754
C=O ketone	1644
C=C aromatic	1614, 1514, 1433
C-O	1289
C-H aromatic	982

υ - wavenumber

^{1}H and ^{13}C NMR data of compound **24** are presented in **APPENDIX VI**.

Characteristic signals of the aromatic protons H-2', H-5', and H-6' appeared at δ_H 7.56, 7.08, and 7.72 ppm, respectively, whereas the corresponding signals of these protons appeared at δ_H 7.41, 7.10, and 7.54 ppm in the non-acetylated parent compound, diosmin (**23**, also in **APPENDIX VI**). Twelve signals characteristic of aliphatic protons (the sugar

moiety) were observed between δ_H 3.65-5.98 ppm, whereas the corresponding signals of these protons appeared between δ_H 3.13-5.03 ppm in the non-acetylated parent compound, diosmin (**23**). Eight singlets between δ_H 1.92-2.43 ppm integrating each for three protons were assigned for -CH_3 protons of acetyl groups, which revealed the formation of the peracetylated derivative **23**.

^{13}C NMR spectrum showed eight signals between δ_C 168.8-170.2 ppm, and eight signals between δ_C 20.6-31.0 ppm, which were assigned to eight C=O of the acetyl groups and to eight -CH_3 groups, respectively.

In ^{13}C NMR spectrum of non-acetylated diosmin (**23**), carbons C-5 and 3' were assigned to δ_C 161.1 and 146.9 ppm, respectively, whereas these carbons were shielded (δ_C 159.8 and 140.1 ppm) in compound **24**. In contrast, carbons C-3 and C-8, in non-acetylated diosmin (**23**), were respectively assigned to δ_C 105.7 and 95.1 ppm, whereas these carbons were deshielded (δ_C 109.0 and 97.8 ppm) in compound **24**.

2.2.15. Rutin peracetate (26)

Structure elucidation of rutin peracetate (**26**, **Figure 35**) was established by IR, NMR (^1H and ^{13}C), HSQC and HMBC. The NMR (^1H and ^{13}C) is in accordance with those reported.[129, 130]

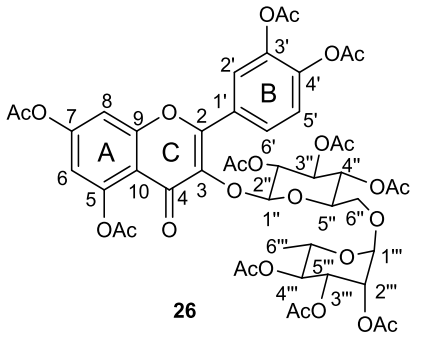


Figure 35 – Rutin peracetate (**26**).

IR spectrum showed two bands at 1754 and 1781 cm^{-1} (C=O ester stretch) suggesting the presence of acetyl groups (**Table 17**).

Table 17 – IR data of compound **26**.

Groups	**υ (cm^{-1})**
	26
C-H aliphatic	2940
C=O ester	1781, 1754
C=O ketone	1628
C=C aromatic	1505, 1477, 1435
C-O	1145

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **26** are presented in **APPENDIX VII**.

Characteristic signals of the aromatic protons H-2', H-5', and H-6' appeared at δ_H 7.91, 7.34, and 7.96 ppm, respectively, whereas the corresponding signals of these protons appeared at δ_H 7.54, 6.85, and 7.56 ppm in the non-acetylated parent compound, rutin (**25**, also in **APPENDIX VII**). Twelve signals characteristic of aliphatic protons (the sugar moiety) were observed between δ_H 3.26-5.43 ppm, whereas the corresponding signals of these protons appeared between δ_H 3.06-5.37 ppm in the non-acetylated parent compound, rutin (**25**). Ten singlets between δ_H 1.60-2.44 ppm integrating each for three protons were assigned for -CH_3 protons of acetyl groups, which revealed the formation of the peracetylated derivative **26.**

^{13}C NMR spectrum showed eight signals between δ_C 167.8-170.2 ppm, and eight signals between δ_C 20.6-21.2 ppm, which were assigned to seven C=O of the acetyl groups and to seven -CH_3 groups, respectively.

In ^{13}C NMR spectrum of non-acetylated rutin (**25**), carbons C-5, 7, 3', and 4' were assigned to δ_C 161.3, 164.1, 144.8, and 149.5 ppm, respectively, whereas these carbons were shielded (δ_C 150.2, 153.9, 141.8, and 144.1 ppm) in compound **26**, In contrast, carbons C-6 and C-8, in non-acetylated rutin (**25**), were respectively assigned to δ_C 98.8 and 100.8 ppm, whereas these carbons were deshielded (δ_C 113.4 and 109.1 ppm) in compound **26**.

The assignments of the carbon atoms directly bonded to proton atoms were achieved from HSQC experiments and the chemical shifts of the carbon atoms not directly bonded to proton atoms were deduced from HMBC correlations (**Figure 36**).

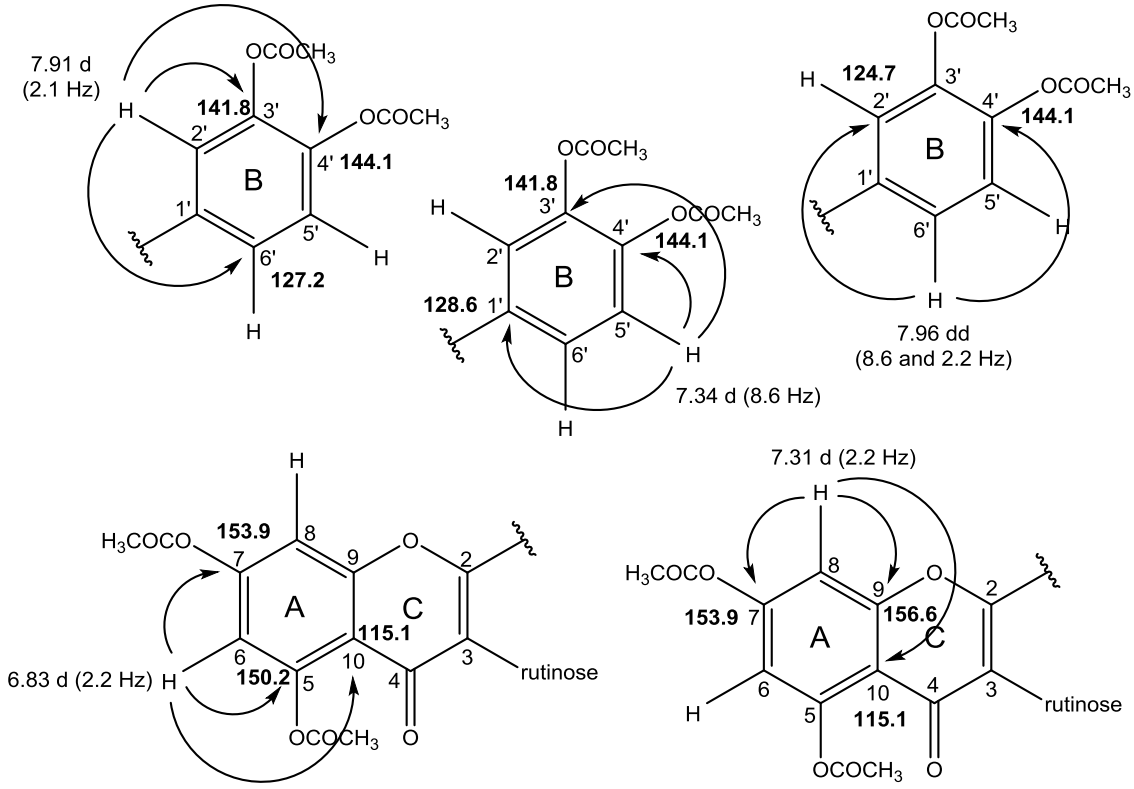


Figure 36 - Main connectivities found in HMBC for compound **26**.

2.2.16. Naringin persulfate (27)

Structure elucidation of naringin persulfate (**27**, **Figure 37**) was established for the first time by IR, NMR (^{1}H and ^{13}C), HSQC, HMBC, and high resolution mass spectrometry (HRMS).

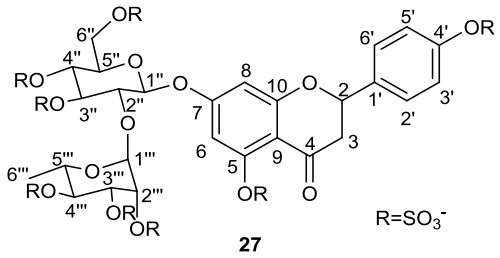


Figure 37 – Naringin persulfate (**27**).

IR spectrum showed bands at 1254 cm^{-1} (S=O), 1052 cm^{-1} (C-O-S), and 810 cm^{-1} (S-O) suggesting the presence of sulfate groups (**Table 18**).

Table 18 – IR data of compound **27**.

Groups	**υ (cm^{-1})**
	27
C=O ketone	1637
C=C aromatic	1518
S=O	1254
C-O-S	1052
S-O	810

υ - wavenumber

^{1}H and ^{13}C NMR data for compound **27** are presented in **APPENDIX II**.

Characteristic signals of the aromatic protons H-2', H-5' and H-3', H-6' appeared as duplets at δ_H 7.58 and 7.17 ppm, respectively, whereas the corresponding signals of these protons appeared at δ_H 7.33, and 6.80 ppm in the non-sulfated parent compound, naringin (**8**, also in **APPENDIX II**). Twelve signals characteristic of aliphatic protons (the sugar moiety) were observed between δ_H 3.71-5.31 ppm, whereas the corresponding signals of these protons appeared between δ_H 3.15-5.14 ppm in the non-sulfated parent compound, naringin (**8**).

In ^{13}C NMR spectrum of non-sulfated naringin (**8**), carbons C-5 and C-4' were assigned to δ_C 162.9 and 157.9 ppm, respectively, whereas these carbons were shielded (δ_C 157.0 and 155.6 ppm) in compound **27**. In contrast, carbons C-6 and C-8, in non-sulfated naringin (**8**), were respectively assigned to δ_C 96.3 and 95.2 ppm, whereas these carbons were deshielded (δ_C 97.1 and 96.0 ppm) in compound **27**.

The assignments of the carbon atoms directly bonded to proton atoms were achieved from HSQC experiments and the chemical shifts of the carbon atoms not directly bonded to proton atoms were deduced from HMBC correlations (**Figure 38**).

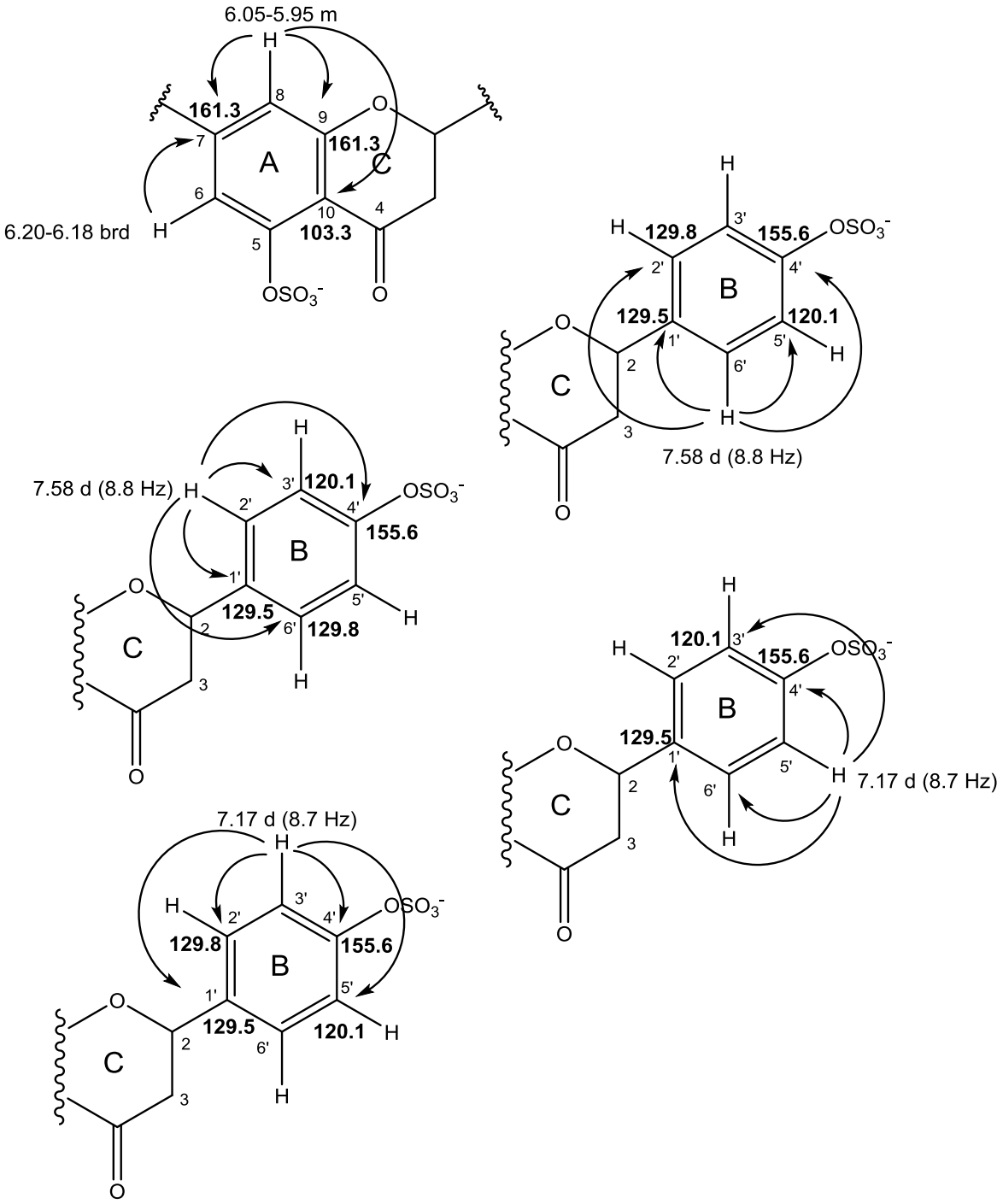


Figure 38 - Main connectivities found in HMBC for compound **27**.

2.3. BIOLOGICAL ACTIVITIES

2.3.1. Antitumor activity

Flavonoids and xanthones are privileged structures, what explains the wide range of biological activities that they display. Natural products such as xanthones and flavonoids are well known for their antitumor properties.[82, 131]

Acetylated derivatives possessing antitumor activity have been described in the literature.[132-135]

Mangiferin heptaacetate (**6**), diosmin peracetate (**26**) and rutin peracetate (**27**) were evaluated *in vitro* for growth-inhibitory effect on three human tumor cell lines: A375-C5 (malignant melanoma IL-1 insensitive), MCF-7 (breast adenocarcinoma), and NCI-H460 (bronchioalveolar carcinoma) using a sulforhodamine B assay (**Table 19**), a methodology that determines cell density, based on the measurement of cellular protein content. These studies were performed at CESPU by Patrícia Duarte and Patrícia Silva under supervision of Professor Hassan Bousbaa.

A pronounced growth-inhibitory effect was observed for compounds **6** and **26** against the three human tumor cell lines (**Table 19**). Compound **26** was more potent than compound **6** in all the three cell-lines.

Table 19 – Cell growth inhibition activity displayed by compounds **6**, **24**, and **26** on three human tumor cell lines.

Compounds	GI_{50}(μM)		
	A375-C5	**MCF-7**	**NCI-H460**
6	56.12 ± 1.69	87.98 ± 2.73	95.79 ± 5.65
24	>150	>150	>150
26	8.29 ± 2.98	18.98 ± 5.12	17.57 ± 2.46

Doxorrubicin was used as positive control. GI_{50} - concentration for 50% of maximal inhibition of cell proliferation.

Compounds **6** and **26**, the most promising compounds, were further tested against three human tumor lines of glioblastoma. Compound **26** was also shown to have potent inhibitory effect on the human glioblastoma cell lines tested (**Table 20**).

Table 20 – Cell growth inhibition activity of compounds **6** and **26** on three human glioblastoma cell lines.

Compounds	GI_{50}(μM)		
	U251	**U373**	**U87MG**
6	>150	>150	>150
26	21.57 ± 3.03	18.77 ± 2.01	13.13 ± 0.68

Doxorrubicin was used as positive control. GI_{50} - concentration for 50% of maximal inhibition of cell proliferation.

Compound **26** was the only compound that showed potent inhibitory effects against all the human tumor cell lines tested. These results stimulate the future synthesis of other polyphenolic compounds to establish other structure-activity relationships. Although the number of derivatives investigated was very reduced, it is possible to infer that the position of the sugar moiety seems to be critical for the inhibitory activity.

2.3.2. Anticoagulant activity

4'-Naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide persulfate (**16**), 3,6-bis(1-(2-(2-amino-3,4,6-tri-*O*-sulfate-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**22**), and naringin persulfate (**27**) were screened *in vitro* for anticoagulant activity by the three classical coagulation times: APTT, PT, and TT (**Figure 39**). These clotting assays are commonly used in clinical laboratory for monitoring anticoagulant drugs in patients and in research laboratories to test anticoagulant activity of new compounds.

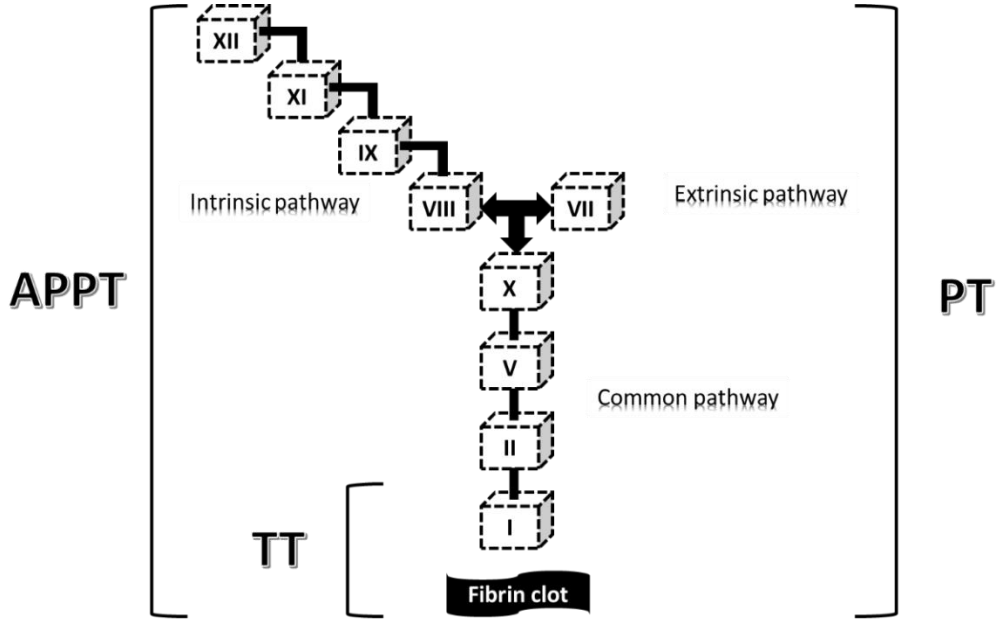


Figure 39 - Representation of the coagulation cascade and the classical clotting assays.

APTT is one of the most used clotting assays and measures the activity of a large number of factors of the intrinsic and common pathways of the coagulation (**Figure 39**). This assay is performed using plasma previously treated with sodium citrate to prevent coagulation. A negative charged activator is added to the citrated plasma and addition of calcium initiates the coagulation process. The APPT is measured after the activation of factor XII until the formation of a stable clot.

PT is widely used in health care to diagnose the risk of bleeding and to monitor oral anticoagulation therapy.[136] The PT test specifically measures the activity of coagulation factors of the extrinsic and common pathway (**Figure 39**). The test involves re-calcification of plasma once the sample has been incubated with the tissue factor source (apoprotein and phospholipid). The PT is measured after the activation of FXa until the formation of a stable clot.

TT measures the time required for formation of a stable clot, following addition of thrombin to citrated plasma.[137] The TT test is commonly used to diagnose deficiencies in fibrinogen (**Figure 39**) and measures the time since the addition of thrombin until the formation of a stable clot.

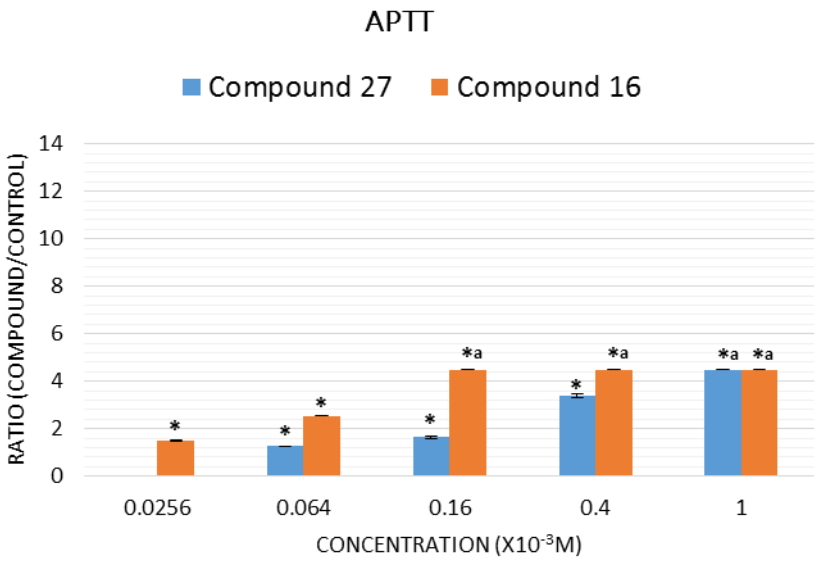
i) BILE ACID CONJUGATE

The effects of persulfated naringin-deoxycholic acid conjugate **16** and non-conjugated naringin persulfate (**27**) on APTT, PT, and TT are represented in **Figure 40**. The anticoagulant activity of the parent non-sulfated conjugate (**15**) and non-sulfated naringin

(**8**) was not possible to test due to their low water solubility. The % of dimethylsulfoxide (DMSO) necessary to solubilize the non-sulfated molecules resulted in precipitation of plasma proteins.

Compound **16** showed potent anticlotting activity (**Table 21**). In the presence of 1 mM of compound **16**, the formation of the fibrin clot was completely inhibited i.e., was not observed even after 180 s (APTT), 120 s (PT), and 240 s (TT) (**Figure 40**). At 160 μM, the clot formation after the activation of factor XII (APTT) was still inhibited and the TT was prolonged above the double (**Figure 40**). The concentration required to double the APPT ($APTT_2$) was around 40 μM (**Table 21**). Additionally, compound **16** showed better anticoagulant activity when compared with other sulfated phenolic compounds previously synthesized in LQOF.[74, 75, 138]

Non-conjugated naringin persulfate (**27**) also affected the APTT, PT, and TT in a dose-dependent manner (**Figure 40**), however higher concentrations were necessary to achieve the same ratio, comparing with compound **16**. Complete inhibition of APTT was only observed at 1 mM and the concentration able to double the APTT was around 200 μM (**Table 21**). So, conjugate **16** is more potent anticoagulant than compound **27**. The conjugation not only retained but also increased the anticoagulant activity of compound **27**. This result was expected because hydroxyl groups of the steroid were also sulfated.



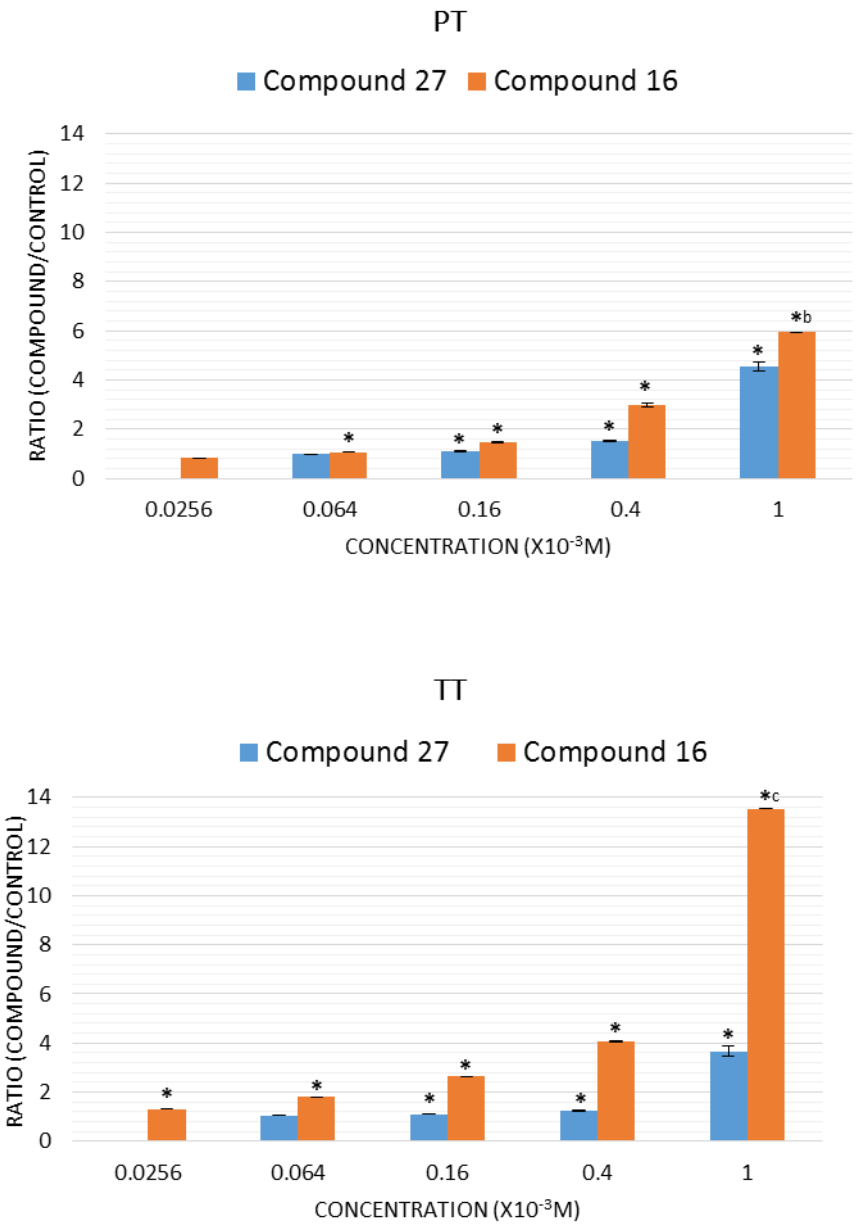


Figure 40 - Dose-dependent effects of compounds **16** and **27** on APTT, PT, and TT clotting assays using human pooled plasma, expressed as ratio of clotting time in the presence/absence of compound. [a] clotting time values greater than 180s, [b] clotting time values greater than 120s, [c] clotting time values greater than 240s, * $P < 0.05$

Table 21 - Effects of sulfated compounds **16** and **27** on blood coagulation.

	16	**27**
APTT inhib.[a] ($x10^{-3}$M)	0.16	1
$APTT_2$ ($x10^{-3}$M)	0.0442 ± 0.0002	0.209 ± 0.005
PT inhib.[b] ($x10^{-3}$M)	1	-
PT_2 ($x10^{-3}$M)	0.222 ± 0.050	-
TT inhib.[c] ($x10^{-3}$M)	1	-
TT_2 ($x10^{-3}$M)	0.0864 ± 0.0007	-

Tthe synthesis and anticoagulant activity of sulfated derivatives of deoxycholic acid (**9**) and 4'-naringin acetic acid (**12**) would be interesting to perform in order to compare with the conjugated compound **16**.

ii) TRIAZOLE LINKED XANTHONOSIDE

The effects of 3,6-bis(1-(2-(2-amino-3,4,6-tri-*O*-sulfate-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (**22**) on APTT, PT, and TT are represented in **Figure 41**. The anticoagulant activity of non-sulfated compound **21** was not possible to test due to their low water solubility. The % of DMSO necessary to solubilize the non-sulfated compound resulted in precipitation of plasma proteins.

Prolongation of APTT, PT, and TT in the presence of compound **22** was observed in a dose-dependent manner. However, PT and TT were less affected than APTT. Sulfated derivative **22** exhibited an $APTT_2$ of 129 ± 3 μM, PT_2 of 739 ± 10 μM, and TT_2 of 230 ± 3 μM. These values were higher than the previous obtained for the non-triazole 3,6-(*O*-*β*-glucopyranosyl) xanthone persulfate (**2**).[75]

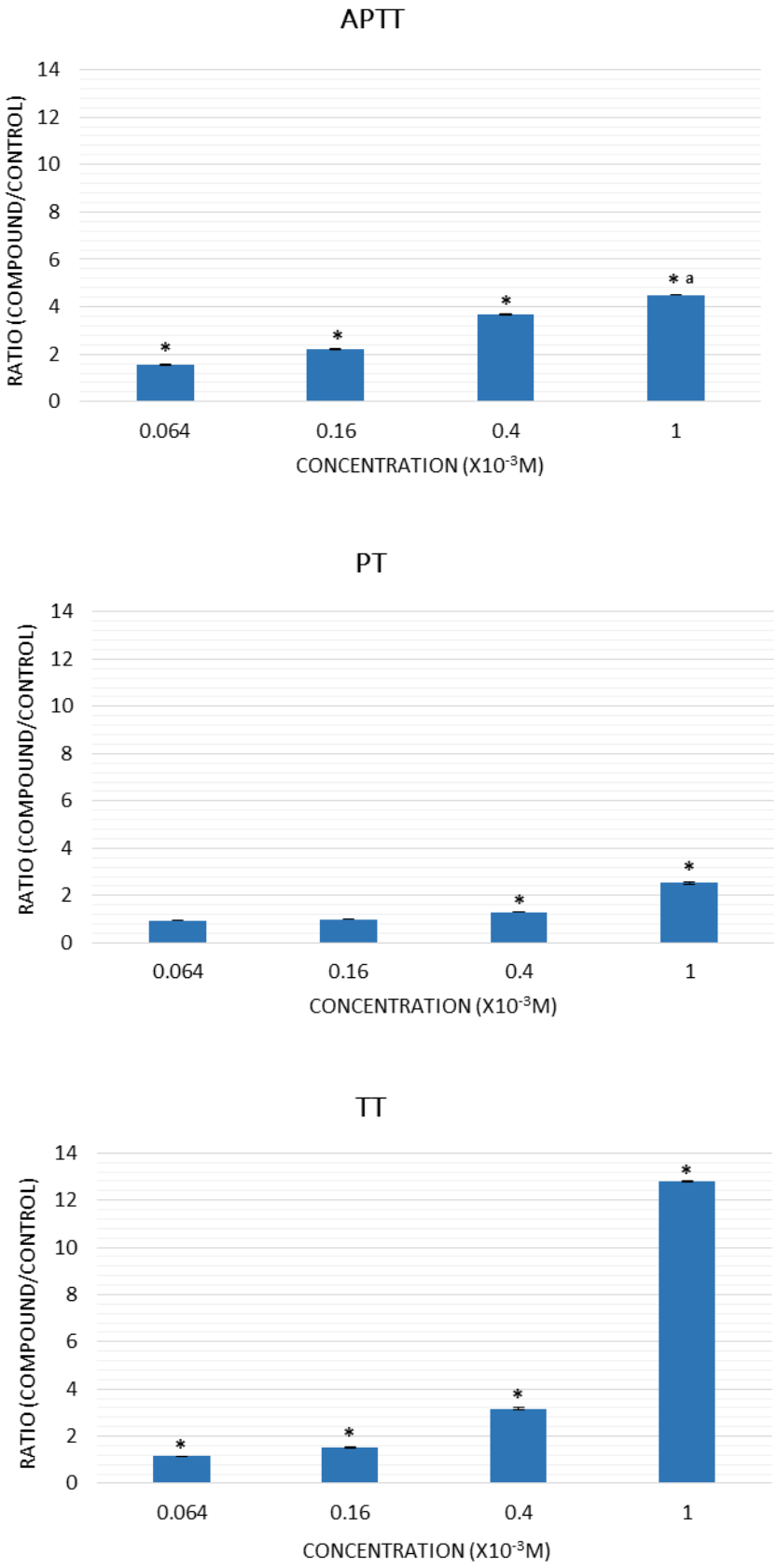


Figure 41 - Dose-dependent effects of compound **22** on APTT, PT, and TT clotting assays using human pooled plasma, expressed as ratio of clotting time in the presence/absence of compound. [a] clotting time values greater than 180s. * $P < 0.05$.

In contrast to bile acid conjugation strategy, the triazole introduction strategy seems to decrease the *in vitro* anticlotting activity, although it is worthy to perform permeability studies with Ussing chamber to test if compound **22** is able to cross the epithelial membrane.

CHAPTER 3 - EXPERIMENTAL SECTION

3.1. GENERAL MATERIALS AND METHODS

Commercial available reagents were purchased from Sigma Aldrich Co. and from Fluka. 3,6-Bis(prop-2-yn-yloxy)-9*H*-xanthen-9-one was synthesized in Laboratório de Química Orgânica e Farmacêutica da Faculdade de Farmácia, Universidade do Porto. Solvents used were products pro analysis or HPLC grade of the firms Sigma-Aldrich and Fluka.

Reactions were controlled by TLC using Merck silica gel 60 (GF254) plates. Compounds were visually detected by absorbance at 254 and/or 365 nm and ferric chloride 10% in MeOH or ninhydrin 3 mg/ml in EtOH following heat activation. MW reactions were performed using a MicroSYNTH 1600 from Millestone (ThermoUnicam, Portugal) synthesizer in sealed and open reaction vessels.

Purification of compounds was carried out either by flash chromatography using Fluka silica gel 60 (0.04-0.063 mm) or preparative TLC using Merck silica gel 60 (GF_{254}) plates. The qualitative purity of the synthesized compounds was determined by TLC using two different mobile phases.

Melting points were obtained in a Köfler microscope and are uncorrected. IR spectra were measured on an ATI Mattson Genesis series FTIR (software: WinFirst v.2.10) spectrophotometer in KBr microplates (cm^{-1}). ^{1}H and ^{13}C NMR spectra were performed in University of Aveiro, Department of Chemistry and were taken in $CDCl_3$ and DMSO-d_6, at room temperature, on Bruker Avance 300 instrument (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C). ^{13}C NMR assignments were made by 2D HSQC and HMBC experiments (long-range C, H coupling constants were optimized to 7 and 1 Hz). Chemical shifts are expressed in ppm values relative to tetramethylsilane (TMS) as an internal reference. Coupling constants are reported in hertz (Hz). HRMS mass spectra were measured on a APEX III mass spectrometer, recorded as ESI (Electrospray) made in Centro de Apoio Científico e Tecnolóxico á Investigation (CACTI, University of Vigo, Spain).

Six human tumor cell lines were used to study antitumor activity: MCF-7 ER(+) (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and A375-C5 (melanoma), U251 (glioblastoma astrocytoma), U373 (glioblastoma astrocytoma) and U87MG glioblastoma astrocytoma).

APTT, PT, and TT tests were performed using an STA Evolution coagulometer. The following commercial reagents were used: 00308 (Stago, França) for the APTT, 00665 (Stago, França) for the PT and 00611 (Stago, França) for the TT.

3.2. SYNTHESIS

3.2.1. Synthesis of mangiferin peracetate (6)

Mangiferin (**5**, M3547, 0.2 g, 0.5 mmol) and iodine (0.009 g, 0.07 mmol) were mixed in acetic anhydride (7 mL) and the reaction was kept under MW irradiation (400W) at 130 ˚C for 15 minutes. After cooling, a saturated solution of sodium thiosulfate was added to transform iodine (dark yellow) into iodide (yellow). The crude product was extracted with CH_2Cl_2 and the organic layer was extracted with a saturated solution of $NaHCO_3$ twice, dried with anhydrous Na_2SO_4, and filtered. The solvent was evaporated under reduced pressure and the oil obtained was dissolved in ethyl acetate and insolubilized with petroleum ether 60-80˚C to afford 1,3,6,7-tetra-*O*-acetyl-2-*C*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-9*H*-xanthen-9-one (**6**) as a yellow solid (0.294 g, 0.39 mmol, 78% yield). mp 143-147 ˚C (petroleum ether 60-80˚C); IR (KBr) υmax: 2937, 1781, 1754, 1664, 1618, 1459, 1370, 1172 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz) *δ*: 8.00 (1H, *s*, H-8), 7.39 (1H, *s*, H-5), 7.25 (1H, *s*, H-4), 5.73 (1H, *t*, *J*=9.7 Hz, H-2'), 5.35-5.14 (2H, *m*, H-3´ and H-4'), 4.86 (1H, *dd*, *J*=4.9 and 10.1 Hz, H-1´), 4.42 (1H, *dd*, *J*=4.2 and 12.6 Hz, H-6'b), 4.05-3.98 (1H, *d*, *J*=12.6 Hz) H-6'a), 3.84-3.80 (1H, *m*, H-5'), 2.53 (3H, *s*, $COCH_3$), 2.49 (3H, *s*, $COCH_3$), 2.34 (3H, *s*, $COCH_3$), 2.32 (3H, *s*, $COCH_3$), 2.07 (3H, *s*, $COCH_3$), 2.06 (3H, *s*, $COCH_3$), 2.03 (3H, *s*, $COCH_3$) 1.80 (3H, *s*, $COCH_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) *δ*: 174.0 (C-9), 170.6 ($OCOCH_3$), 170.5 ($OCOCH_3$), 170.3 ($OCOCH_3$), 169.6 ($OCOCH_3$), 169.2 ($OCOCH_3$), 168.5 ($OCOCH_3$), 168.0 ($OCOCH_3$), 167.7 ($OCOCH_3$), 167.2 ($OCOCH_3$), 157.4 (C-4a), 154.4 (C-3), 152.9 (C-1 and C-6), 147.7 (C-10a), 139.4 (C-7), 120.8 (C-8), 120.1 (C-8a), 118.3 (C-2), 112.7 (C-5), 112.1 (9a), 111.7 (C-4), 76.6 (C-5'), 74.4 (C-3'), 72.5 (C-1'), 69.4 (C-2'), 68.1 (C-4'), 61.9 (C-6'), 21.4 ($OCOCH_3$), 21.3 ($OCOCH_3$), 21.1 ($OCOCH_3$), 20.8 ($OCOCH_3$), 20.7 ($OCOCH_3$), 20.5 ($OCOCH_3$), 20.4 ($OCOCH_3$), 20.3 ($OCOCH_3$).

3.2.2. Synthesis of mangiferin heptaacetate (7)

Mangiferin (**5**, M3547, 0.2 g, 4.7 mmol) was mixed with acetic anhydride (10 mL) and the mixture was heated at 130 ˚C for 3.5 hours. After cooling, the solution obtained was poured into ice and a saturated solution of $NaHCO_3$, and extracted with CH_2Cl_2. Organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude oil obtained was further purified using flash chromatography (SiO_2; hexane:ethyl acetate, 7:3) to give 1-hydroxy-3,6,7-tri-*O*-acetyl-2-*C*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-9*H*-xanthen-9-one (**7**) as a light yellow solid (0.0378 g, 0.053 mmol, 11% yield). mp 160-162 ˚C (hexane/ethyl acetate); IR (KBr) υmax: 3443, 2919, 2850, 1779,

1746, 1647, 1619, 1467, 1372, 1229 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz) *δ*: 13.25 (1H, *s*, 1-OH), 8.06 (1H, *s*, H-8), 7.42 (1H, *s*, H-5), 6.76 (1H, *s*, H-4), 5.68 (1H, *brs*, H-2'), 5.35 (1H, *t*, *J*= 9.4 Hz, H-3'), 5.23-5.17 (2H, *m*, H-4' and H-1'), 4.40 (1H, *d*, *J*= 11.6 Hz, H-6'b), 4.04-4.00 (1H, *m*, H-6'a), 3.85-3.81 (1H, *m*, H-5'), 2.44 (3H, *s*, $COCH_3$), 2.35 (3H, *s*, H- $COCH_3$), 2.34 (3H, *s*, $COCH_3$), 2.07 (3H, *s*, $COCH_3$), 2.05 (3H, *s*, $COCH_3$), 2.02 (3H, *s*, $COCH_3$) 1.78 (3H, *s*, $COCH_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) *δ*: 180.4 (C-9), 170.5 (O*C*OCH_3), 170.2 (O*C*OCH_3), 169.6 (O*C*OCH_3), 169.2 (O*C*OCH_3) 169.4 (O*C*OCH_3), 168.0 (O*C*OCH_3), 167.1 (O*C*OCH_3), 161.3 (C-1), 156.7 (C-4a), 153.9 (C-3),148.4 (C-6 and C10a), 139.4 (C-7), 120.1 (C-8), 119.9 (C-8a) 118.4 (C-2), 112.9 (C-5), 110.6 (C-4), 106.0 (C-9a), 76.5 (C-5'), 74.5 (C-3'), 73.3 (C-1'), 70.4 (C-4'), 68.3 (C-2'), 62.1 (C-6'), 22.7 (CO*C*H_3), 21.3 (CO*C*H_3), 20.7 (CO*C*H_3), 20.7 (CO*C*H_3), 20.6 (CO*C*H_3), 20.5 (CO*C*H_3), 20.4 (CO*C*H_3).

3.2.3. Synthesis of naringin-di-deoxycholate (10)

DOCA (**9**, D2510, 0.338 g, 0.86 mmol, 1 eq.) and TBTU (Fluka 12806, 0.277 g, 0.86 mmol, 1 eq.) were dissolved in tetrahydrofuran (THF) and TEA (1.72 mmol, 0.24 mL, 2 eq.) was added. After 15 minutes, naringin (**8**, N1376, 0.5 g, 0.86 mmol) was added. The mixture was kept under a nitrogen atmosphere at room temperature for 72 hours. The solution obtained was poured into ice and the solid formed was filtered and purified by flash chromatography (SiO_2; $CHCl_3$:MeOH:HCOOH, 8:2:0.1) and then by preparative TLC (SiO_2; $CHCl_3$:MeOH:HCOOH, 8:2:0.1) to give the ester derivate **10** (0.025 g, 0.0188 mmol, 2.2%) as white solid. mp 182-185 ˚C (H_2O; IR (KBr) υmax: 3415, 2933, 2865, 1737, 1642, 1448, 1374, 1087; ^{1}H NMR (DMSO-d_6, 300.13 MHz) *δ*: 12.03 (1H, *s*, 5-OH naringin), 7.56 (2H, *d*, *J*=8.1 Hz, H-2', H-6' naringin), 7.17 (2H, *d*, *J*=8.5 Hz, H-3', H-5' naringin), 6.15-6.09 (2H, *m*, H6, H-8 naringin), 5.70-5.61 (1H, *m*, H-2 naringin), 5.47 (1H, *d*, *J*=4.6 Hz, OH), 5.42 (2H, *d*, *J*=5.2 Hz, OH), 5.20-5.16 (1H, *m*, H-1''' neohesperidoside), 5.09 (1H, *brd*, H-1'' neohesperidoside), 4.77-4.74 (1H, *m*, OH), 4.69 (1H, *d*, *J*=4.3 Hz, OH), 4.50-4.48 (3H, m, OH), 4.26 (2H, d, J=3.9 Hz, H-12), 4.18-4.16 (1H, m, OH), 4.07-4.00 (1H, m, OH), 3.82 (1H, *d*, *J*=2.9 Hz, 3-OH DOCA), 3.73-3.67 (6H, *m*, H-neohesperidoside, H-3 DOCA), 3.51-3.28 (2H, *m*, H-neohesperidoside, under water), 3.23-3.16 (4H, *m*, H-neohesperidoside), 2.89-2.87 (1H, *m*, H-3_{eq} naringin), 2.83-2.81 (1H, *m*, H-3_{ax} naringin), 2.28-2.18 (2H, H-23 DOCA), 1.82-1.24 (56H, *m*, H-steroidal DOCA), 1.17 (3H, *d*, *J*=6.1 Hz, 6'''-CH_3 neohesperidoside), 0.99 (6H, *d*, *J*=5.7, 21-CH_3), 0.85 (6H, *s*, 19-CH_3), 0.63 (3H, *s*, 18-CH_3 DOCA), 0.56 (3H, *s*, 18-CH_3 DOCA); ^{13}C NMR (DMSO-d_6, 75.47 MHz) *δ*: 197.0 (C-4 naringin), 173.2 (C-24 DOCA), 172.2 (C-24 DOCA), 163.0 (C-5 naringin), 162.8 (C-7 naringin), 150.7 (C-4' naringin), 136.2 (C-1' naringin), 128.0 (C-2', C-6' naringin), 122.0 (C-3', C-5' naringin), 100.7

(C-1''), 97.0 (C-1'' neohesperidoside, C-6/8 naringin), 95.0 (C-6/8 naringin) 77.0 (C-2 naringin), 71.1 (C-neohesperidoside, C-12 DOCA), 70.4 (C-neohesperidoside), 70.0 (C-neohesperidoside, C-3 DOCA), 68.0 (C-neohesperidoside), 47.5 (C-14 DOCA), 47.4 (C-14 DOCA), 46.2 (C-17 DOCA), 46.1, 46.0 (C-13 DOCA), 41.6 (C-5 DOCA, C-3 naringin), 40.3, 40.1, 39.8, 39.5, 39.2 (C-DOCA), 36.3 (C-8 DOCA), 35.7 (C-4 DOCA), 35.2 (C-20 DOCA), 35.0 (C-1), 33.9 (C-10 DOCA), 33.0, 30.8 (C-23 DOCA), 30.6 (C-23 DOCA), 30.3 (C-2 DOCA), 28.6 (C-11 DOCA), 27.3 (C-6 DOCA), 27.0 (C-16 DOCA), 26.1 (C-7 DOCA), 23.5 (C-15 DOCA), 23.1 (C-19 DOCA), 18.1 (C-6''' neohesperidoside), 17.0 (C-21 DOCA), 16.7 (C-21 DOCA), 12.5 (C-18 DOCA), 12.4 (C-18 DOCA).

3.2.4. Synthesis of methyl 4'-naringin acetate (11)

Naringin (**8**, N1376, 1 g, 1.72 mmol) was dissolved in DMF (20 mL) and K_2CO_3 (0.285 g, 2.06 mmol, 1.2 eq.) was added. After 10 minutes, methyl bromoacetate (253 µL, 2.75 mmol, 1.6 eq.) was added. The mixture was kept at room temperature for 32 hours. The solution obtained was evaporated under reduced pressure and the solid was purified by flash chromatography (SiO_2:$CHCl_3$:acetone) to give methyl 4'-(7-[[2-*O*-(6-Deoxy-*α*-L-mannopyranosyl)-*β*-D-glucopyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-oxy)) acetate (**11**) as an orange solid (0.206 g; 0.32 mmol; 18% yield). mp 166-170 ˚C ($CHCl_3$:acetone); IR (KBr) υmax: 3420, 2922, 1754, 1632, 1511, 1439, 1216, 1176, 1078, 832, 668; ^{1}H NMR (DMSO-d_6, 300.13 MHz) *δ*: 12.05 (1H, *s*, 5-OH), 7.46 (2H, *dd*, *J*=2.4 and 8.9 Hz, H-2' and H6'), 7.02-6.97 (2H, *m*, H3' and H-5'), 6.20 (1H, *d*, *J*=2.5 Hz, H-8), 6.13-6.08 (1H, *m*, H-6), 5.60-5.52 (1H, *m*, H-2), 5.15-5.10 (3H, *m*, H-1'', H-1''' and OH), 4.91-4.87 (4H, *m*, OHs), 4.83 (2H, *s*, O-CH_2), 3.74-3.64 (3H, *m*, H-5''', H-2''', and H-6''a), 3.71 (3H, *s*, O-CH_3), under water (4H, *m*, H-2'', H-3'', H5'' and H-6''b), 3.23-3.15 (3H, *m*, H-3''', H-4''' and H-4''), 2.82-2.73 (2H, m, H-3), 1.16 (3H, *d*, *J*=6.2 Hz, 6'''-CH_3); ^{13}C NMR (DMSO-d_6, 75.47 MHz) *δ*: 197.2 (C-4), 169.2 (*C*OOCH_3), 164.8 (C-7), 163.0 (C-5), 162.8 (C-9), 157.9 (C-4'), 128.5 (C-1'), 128.4 (C-2'), 128.2 (C-6'), 115.1 (C-3' and C-5'), 103.4 (C-10), 100.5 (C-1''), 97.3 (C-1'''), 96.4 (C-6), 95.2 (C-8), 79.2 (C-2), 77.1 (C-2''), 76.9 (C-5''), 76.1 (C-3''), 71.8 (C-4'''), 70.5 (C-2'''), 70.4 (C-3'''), 69.6 (C-4''), 68.3 (C-5'''), 65.4 (4'-OCH_2), 60.5 (C-6''), 51.9 (O*C*H_3), 45.4 (C-3), 18.1 (C-6''').

3.2.5. Synthesis of 4'-naringin acetic acid (12)

Compound **11** (0.207 g, 0.316 mmol) was dissolved in MeOH (4 mL) and sodium methoxide (0.5 M solution in MeOH, 3 mL, 1.5 mmol) was added. The mixture was kept at room temperature for 5 hours. After completion of the reaction, the solvent was evaporated

under reduced pressure and the product neutralized using a strong cationic exchange solid phase extraction column. H_2O was evaporated under reduced pressure to give (7-[[2-*O*-(6-deoxy-*α*-L-mannopyranosyl)-*β*-D-glucopyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-oxy) acetic acid (**12**) as a dark yellow solid (0.199 g; 0.313 mmol; 99% yield). mp 215 ˚C dec (H_2O); IR (KBr) υmax: 3438, 2919, 1735, 1628, 1512, 1424, 1348, 1215, 1177, 1128, 1072, 813; ^{1}H NMR (DMSO-d_6, 300.13 MHz) *δ*: 13.89 (1H, *s*, COOH), 12.05 (1H, *s*, 5-OH), 7.44 (2H, *dd*, *J*=2.4 and 8.8 Hz, H-2' and H-6'), 6.93 (2H, *d*, *J*=8.8 Hz, H-3' and H-5'), 6.16-6.08 (2H, *m*, H-6 and H-8), 5.61-5.53 (1H, *m*, H-2), 5.17-5.05 (4H, *m*, H-1'', H-1''', and 2OH), 4.62 (1H, *d*, *J*=3.5 Hz, OH), 4.57 (1H, *d*, *J*=4.4 Hz, OH), 4.51 (1H, *m*, OH), 4.43-4.38 (1H, *m*, OH), 3.74-3.64 (2H, *m*, H-6''a, H-5'''), 3.49-3.16 (1H, H-6''b), 3.25-3.16 (3H, *m*, H-2''', H-3''', and H-4'''), 2.82-2.71 (2H, *m*, H-3), 1.16 (3H, *d*, *J*=6.2 Hz, 6'''-CH_3); ^{13}C NMR (DMSO-d_6, 75.47 MHz) *δ*: 197.2 (C-4), 170.1 (C=O), 164.8 (C-7), 163.3 (C-5), 162.8 (C-9), 157.9 (C-4'), 128.5 (C-1'), 128.4 (C-2'), 128.3 (C-6'), 115.0 (C-3' and C-5'), 100.5 (C-1''), 103.3 (C-10), 97.4 (C-1'''), 96.3 (C-6), 95.2 (C-8), 78.9 (C-2), 77.1 (C-2''), 76.9 (C-5''), 71.8 (C-4'''), 70.5 (C-2'''), 70.4 (C-3'''), 69.6 (C-4''), 68.3 (C-5'''), 65.3 (4'-OCH_2), 60.5 (C-6''), 42.0 (C-3), 18.1 (6'''-CH_3).

3.2.6. Synthesis of succinimido deoxycholate (13)

DOCA (**9**, D2510, 1 g, 2.12 mmol) was dissolved in THF (50 mL) and DCC (0.745 g, 3.2 mmol, 1.7 eq./COOH) and NHS (0.416 g, 3.2 mmol, 1.7 eq./COOH) were added. The mixture was kept under nitrogen atmosphere, at room temperature, overnight. After completion of the reaction, insolubilized dicyclohexylurea was removed by filtration. The filtrate was concentrated under reduced pressure, hexane was added, and 2,5-dioxopyrrolidin-1-yl (*R*)-4-((3*R*,5*R*,8*R*,9*S*,10*S*,12*S*,13*R*,14*S*,17*R*)-3,12-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[a]phenanthren-17-yl)pentanoate (**13**) insolubilized as a white solid (1.1 g, 2.25 mmol, 88% yield). IR (KBr) υmax: 3432, 2938, 2863, 1811, 1781, 1741, 1448, 1207, 1066, 1043, 994, 648 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz) *δ*: 4.00 (1H, *t*, *J*= 2.8, 3-OH), 3.77-3.73 (1H, *m*, H-12), 3.98-3-56 (1H, *m*, H-3), 2.87-2.82 (4H, *m*, CH_2-CH_2, NHS), 2.67-2.55 (2H, *m*, H-23a and H-23b), 1.94-1.05 (32H, *m*, H-steroidal), 1.01 (3H, *d*, *J*=6.1 Hz, 21-C*H*$_3$), 0.91 (3H, *s*, 19-C*H*$_3$), 0.70 (3H, *s*, 18-C*H*$_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) *δ*: 169.1 (C-24), 169.2 (C=O, NHS), 73.1 (C-12), 71.8 (C-3), 48.2 (C-14), 47.1 (C-17), 46.6 (C-13), 42.1 (C-5), 36.4 (C-8), 36.0 (C-4), 35.2 (C-20), 34.9 (C-1), 34.1 (C-10), 33.7 (C-9), 30.5 (C-2, C-22, C-23), 28.0 (C-11), 27.4 (C-6), 27.1 (C-16), 26.1 (C-7), 25.6 (*C*H_2-*C*H_2, NHS), 23.6 (C-15), 23.2 (C-19), 17.3 (C-21), 12.7 (C-18).

3.2.7. Synthesis of *N*-deoxycholylethylenediamine (14)

Succinimido deoxycholate (**13**, 0.5 g, 1.02 mmol) was dissolved in DMF (2.5 mL) and EDA (5 mL) was added. The mixture was kept at room temperature for 24 hours. The solution obtained was poured into ice and (*R*)-*N*-(2-aminoethyl)-4-((3*R*,5*R*,8*R*,9*S*,10*S*,12*S*,13*R*,14*S*,17*R*)-3,12-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[a]phenanthren-17-yl)pentanamide (**14**) insolubilized as a white solid (0.361 g, 0.831 mmol, 81% yield). mp 120-123 ˚C (H_2O); IR (KBr) υmax: 3628, 2926, 2861, 1694, 1628, 1558, 1506, 1447, 1374, 671; ^{1}H NMR (DMSO-d_6, 300.13 MHz) *δ*: 7.72 (1H, *t*, *J*= 10.9, NH), 4.49 (1H, *s*, 3-OH), 4.21 (1H, *brd*, H-12), 3.79 (1H, *s*, H-3), 3.01 (4H, *q*, *J*=6.3 and 12.2, CH_2-CH_2), 2.13-2.04 (1H, *m*, H-23a), 2.00-1.90 (2H, *m*, H-23), 1.82-0.97 (31H, *m*, H-steroidal, -NH_2), 0.92 (3H, *d*, *J*=6.2 Hz, 21-CH_3), 0.84 (3H, *s*, 19-CH_3), 0.59 (3H, *s*, 18-CH_3); ^{13}C NMR (DMSO-d_6, 75.47 MHz) *δ*: 172.7 (C-24), 71.0 (C-12), 70.0 (C-3), 47.5 (C-14), 46.2 (C-17), 46.0 (C-13), 42.3 (C-5), 36.3 (C-8), 35.7 (C-4), 35.1 (C-1, C-20), 33.8 (C-10), 32.9 (C-9, C-22), 32.5 (C-23), 28.7 (C-2), 28.6 (C-11), 27.2 (C-6), 27.0 (C-16), 26.1 (C-7), 23.5 (C-15), 23.1 (C-19), 17.1 (C-21), 12.5 (C-18).

3.2.8. Synthesis of 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide (15)

Compound **12** (0.123 g, 0.192 mmol) and TBTU (Fluka 12806, 0.059 g, 0.385 mmol, 2 eq.) were dissolved in DMF (7 mL) and TEA (54 µL, 0.385 mmol; 2 eq.) was added. After 15 minutes, compound **14** (0.084 g, 0.192 mmol, 1 eq.) was added. The mixture was kept at room temperature for 48 hours. After the completion of the reaction the mixture was poured into ice and the solid was filtrated and washed with H_2O, aqueous solution of HCl 1M, and then with H_2O to give (4*R*)-4-((3*R*,5*R*,8*R*,9*S*,10*S*,12*S*,13*R*,14*S*,17*R*)-3,12-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[a]phenanthren-17-yl)-N-(2-(2-(4-(7-[[2-*O*-(6-Deoxy-*α*-L-mannopyranosyl)-*β*-D-glucopyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-oxy))acetamido)ethyl)pentanamide (**15**) as a yellow solid (0.149 g, 0.141 mmol, 73% yield). m.p 214-219 ˚C (H_2O); IR (KBr) υmax: 3443, 2924, 1633, 1384, 1176, 1076; ^{1}H NMR (DMSO-d_6, 300.13 MHz) δ: 12.06 (1H, *s*, 5-OH, naringin), 8.17 (1H, *s*, NH-amide), 7.87-7.79 (2H, *m*, H-2'/6', NH amide), 7.49-7.45 (1H, *brd*, H-2'/6' naringin), 7.03-6.99 (2H, *brd*, H-3', H-5'), 6.21-6.10 (2H, *m*, H-6, H-8 naringin), 5.50-5.54 (1H, *m*, H-2 naringin), 5.36-5.32 (1H, m, OH neohesperidoside), 5.18-5.10 (2H, *m*, H-1'', H-1''' neohesperidoside), 4.77-4.53 (6H, m, OH neohesperidoside, 3-OH DOCA, -OCH_2-), 4.19 (1H, *s*, H-12 doca), 3.77-3.69 (4H, *m*, H-neohesperidoside, H-3 DOCA), 3.55-3.35 (6H, m, H-neohesperidoside, under water signal), 3.18-3.15 (4H, *m*, CH_2CH_2 EDA), 2.89-

2.71 (2H, *m*, H-3 naringin), 2.07-1.91 (2H, m, H-23a, H23b DOCA), 1.75-1.14 (28H, *m*, H-steroidal, 6'''-CH_3 neohesperidoside), 0.91 (3H, *d*, *J*=5.9 Hz, 21-CH_3 DOCA) 0.84 (3H, *s*, 19-CH_3 DOCA), 0.59 (3H, *s*, 18-CH_3 DOCA); ^{13}C NMR (DMSO-d_6, 75.47 MHz) δ: 174.3 (C=O amide), 168.6 (C=O amide), 164.8 (C-7), 163.0 (C-5), 128.9 (C-2', C-6' naringin), 115.6 (C-3', C-5', naringin), 100.9 (C-1'', naeohesperidoside), 97.4 (C-1''', neohesperidoside), 96.8 (C-6,8 naringin), 77.4 (C-2 naringin), 76.6 (C-neohesperidoside), 72.3 (C-neohesperidoside), 71.6 (C-neohesperidoside, C-12 DOCA), 70.7 (C-neohesperidoside), 70.5 (C-neohesperidoside, C-3 DOCA), 68.8 (C-neohesperidoside), 67.2 (-OCH_2-), 60.9 (C-6'', neohesperidoside), 47.8 (C-14, DOCA), 46.6 (C-17, DOCA), 46.4 (C-13, C-3 DOCA), 42.0 (C-5, DOCA), 38.5 (CH_2CH_2-EDA), 36.5 (C- DOCA), 36.0 (C-DOCA), 35.4 (C-DOCA), 34.2 (C-DOCA), 33.3 (C-DOCA), 32.9 (C-DOCA), 32.0 (C-DOCA), 31.9 (C-DOCA), 31.6 (C-23 DOCA), 30.4, 28.9 (C-DOCA), 27.6 (C-DOCA), 27.3 (C-DOCA), 26.5 (C-DOCA), 23.9 (C-15, DOCA), 23.5 (C-19, DOCA), 18.4 (6'''-CH_3, naringin), 17.4 (C-21, DOCA), 12.8 (C-18, DOCA).

3.2.9. Synthesis of 4'-naringin (*N*-(2-deoxycholan-24-amidoethyl))acetamide persulfate (16)

Compound **15** (0.138 g, 0.131 mmol) was dissolved in DMA (15 mL) and triethylamine-sulfur trioxide (SO_3:TEA) adduct (2.13 g, 11.8 mmol, 10 eq./OH) was added. The mixture was kept under MW irradiation (200W) at 100 ºC for 2 hours. After cooling, TEA was added until the pH was 8 and the obtained solution was poured into acetone and left at 4°C for 24 hours. The crude oil formed was washed with acetone and ether to remove unreacted adduct and then was dissolved in aqueous solution of 30% NaOAc (2 mL). EtOH was added and the sodium salt of (4*R*)-4-((3*R*,5*R*,8*R*,9*S*,10*S*,12*S*,13*R*,14*S*,17*R*)-3,12-di-*O*-sulfate-10,13-dimethylhexadecahydro-1*H*-cyclopenta[a]phenanthren-17-yl)-*N*-(2-(2-(4-(7-[[2-*O*-(6-deoxy-*α*-L-mannopyranosyl)-*β*-D-glucopyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-oxy)acetamido)ethyl)pentanamide (**16**) insolubilized as a light brown solid that was further purified by dialysis using a Spectra/Por 6 regenerated cellulose MWCO 1000 membrane (0.130 g, 0.066 mmol, 50% yield). m.p. 215-219 ˚C (EtOH); IR (KBr) υmax: 2921, 2863, 1632, 1558, 1240, 1058, 810; ^{1}H NMR (DMSO-d_6, 300.13 MHz) δ: 8.24 (1H, *s*, NH-amide), 7.90 (2H, *brd*, H-2'/6' naringin, NH amide), 7.49 (1H, brd, H-2'/6' naringin), 7.03 (2H, *brd*, H3', H-5' naringin), 6.19-5.98 (2H, *m*, H-6, H-8 naringin), 5.35-5.20 (3H, *m*, H-2 naringin, H-1'', H-1''' neohesperidoside), 4.75-4.68 (2H, *m*, H-neohesperidoside), 4.53-4.42 (6H, *m*, H-12 DOCA, H-neohesperidoside, -OCH_2-), 4.01-3.70 (6H, *m*, H-neohesperidoside, H-3 DOCA), 3.16-3.15 (4H, *m*, CH_2CH_2-EDA, H-

neohesperidoside), 2.72-2.61 (2H, *m*, H-3, naringin), 2.13-2.09 (2H, *m*, H-23a, H-23b, DOCA) 1.90-1.16 (28H, *m*, H-steroidal, 6'''-CH_3 neohesperidoside), 0.94 (*d*, *J*=6.4 Hz, 21-CH_3 DOCA), 0.85 (3H, *s*, 19-CH_3 DOCA), 0.62 (3H, *s*, 18-CH_3 DOCA); ^{13}C NMR (DMSO-d_6, 75.47 MHz) δ: 173.1 (C=O amide), 167.8 (C=O amide), 164.7 (C-7 naringin), 163.4 (C-5 naringin), 131.0 (C-1' naringin), 128.0 (C-2', C-6', naringin), 114.8 (C-3', C-5' naringin), 100.8 (C-1'' neohesperidoside), 97.3 (C-1''' neohesperidoside), 96.0 (C-6,8), 78.0 (C-2 naringin, C-12 DOCA), 76.1 (C-neohesperidoside, C-3 DOCA), 71.5 (C-neohesperidoside), 67.0 (-OCH_2-), 48.2 (C-14 DOCA), 45.7 (C- DOCA), 45.6 (C-3 naringin, C-13 DOCA), 41.9 (C-5 DOCA), 38.4 (CH_2CH_2-EDA), 38.2 (CH_2CH_2-EDA), 35.5 (C-4 DOCA), 35.3 (C-1 DOCA), 33.9 (C-10 DOCA), 33.5-31.6 (C-DOCA), 27.3 (C-6 DOCA), 27.1 (C-16 DOCA), 26.1 (C-7, DOCA), 24.5, 23.6 (C-15, DOCA), 23.2 (C-19, DOCA), 18.0 (C-6''', neohesperidoside), 17.4 (C-21, DOCA), 12.3 (C-18, DOCA).

3.2.10. Synthesis of 3,6-bis(1-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (19)

3,6-Bis(prop-2-yn-yloxy)-9*H*-xanthen-9-one (**17**, 0.183 g, 0.6 mmol) and 2-azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside (**18**, A1750, 0.499 g, 1.20 mmol) were dissolved in THF/H_2O (2:1, 20 mL) and sodium ascorbate (0.475 g, 2.40 mmol) and $Cu_2SO_4.5H_2O$ (0.3 g, 1.20 mmol) were added. The mixture was kept under MW irradiation (500 W) for 30 min at 70°C. After cooling, the reaction mixture was filtered, the THF of the filtrate was evaporated under reduced pressure, and the water suspension was extracted twice with ethyl acetate. A green solid was formed in the aqueous phase and after filtration 3,6-bis(1-(2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)-9*H*-xanthen-9-one (**19**) was separated as an orange solid (0.497 g, 0.437 mmol, 73% yield). mp 239-241 ˚C (EtOAc); IR (KBr) υmax: 2957, 1745, 1660, 1609, 1441, 1373; ^{1}H NMR (DMSO-d_6, 300.13 MHz) δ: 8.15 (1H, *s*, H-triazole), 8.09 (1H, *d*, *J*=8.9 Hz, H-1/8), 7.92 (1H, *d*, *J*=9.1 Hz, NH-amide), 7.34 (1H, *d*, *J*=2.3 Hz, H-4/5), 7.10 (1H, *dd*, *J*=2.3 and 9.0 Hz, H-2/7), 5.33 (2H, *s*, O-CH_2-triazole), 5.07 (1H, *t*, *J*=9.8 Hz, H-5'/5''), 4.85 (1H, *t*, *J*=9.9 Hz, H-3'/3''), 4.70 (1H, *d*, *J*=8.5 Hz, H-1'/1''), 4.60-4.57 (2H, *m*, N-CH_2CH_2-O), 4.21-4.02 (5H, *m*, H-4'/4'', H6'/6''a, H-6'/6''b, N-CH_2CH_2-O), 3.76 (1H, *q*, *J*=9.7, H-2'/2''), 2.02 (3H, *s*, $COCH_3$), 1.97 (3H, *s*, $COCH_3$), 1.91 (3H, *s*, $COCH_3$), 1.71 (3H, *s*, NHOCH_3); ^{13}C NMR (DMSO-d_6, 75.47 MHz) δ: 174.1 (C-9), 170.1 (*C*OCH_3), 169.7 (*C*OCH_3), 169.4 (*C*OCH_3), 169.1 (NH*C*OCH_3), 163.3 (C-3/6), 157.4 (C-4a/10a), 141.5 (CH=*C* triazole), 127.5 (C-1/8), 125.2 (*C*H=C triazole), 115.0 (C-8a/9a), 113.8 (C-2/7), 101.5 (C-4/5), 99.8 (C-1'/1''), 72.5 (C-5'/5''), 70.7 (C-4'/4''), 68.4 (C-3'/3''), 67.2 (N-CH_2CH_2-N),

61.9 (O*C*H_2-triazole), 61.7 (C-6'/6''), 52.8 (C-2'/2''), 49.4 (N-*C*H_2*C*H_2-O), 22.6 (NHCO*C*H_3), 20.5 (CO*C*H_3), 20.4 (CO*C*H_3), 20.3 (CO*C*H_3).

3.2.11. Synthesis of 3,6-bis(1-(2-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)ethyl)-*1H*-1,2,3-triazole-4-yl)methoxy)xanthone (20)

Compound **19** (0.244 g; 0.215 mmol) was suspended in MeOH (8 mL) and MeONa (1 mL) was added. The mixture was kept under stirring at room temperature for 3 hours. The mixture was filtered and 3,6-bis(1-(2-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)-9*H*-xanthen-9-one (**20**) was obtained as a light green solid (0.162 g; 0.183 mmol, 86% yield). m.p. 238-240 ˚C (MeOH); IR (KBr) υmax: 3415, 2919, 1648, 1610, 1444, 1375; ^{1}H NMR (DMSO-d_6, 300.13 MHz) δ: 8.18 (1H, *s*, H-triazole), 8.10 (1H, *d*, *J*=9.0 Hz, H-1/8), 7.70 (1H, *d*, *J*=8.9 Hz, N*H*-amide), 7.35 (1H, *d*, *J*=2.1 Hz, H-4/5), 7.12 (1H, *dd*, *J*=2.1 and 12 Hz H-2/7), 5.33 (2H, *s*, OCH_2-triazole), 5.08 (1H, *brd*, OH), 5.01 (1H, *brd*, OH), 4.60-4.57 (3H, *m*, N-CH_2CH_2-O, OH), 4.33 (1H, *d*, *J*=8.4 Hz, H-1'/1''), 4.12-4.08 (1H, *m*, N-CH_2CH_2-O), 3.87-3.81 (1H, *m*, N-CH_2CH_2-O), 3.73-3.68 (1H, *m*, H-6'/6''), 3.49-3.40 (1H, m, H-2'/2''), 3.29-3.25 (1H, *m*, H-4'/4''), 3.18-3.04 (2H, *m*, H3'/3'' and H-5'/5'') 1.76 (3H, *s*, NHCOCH_3); ^{13}C NMR (DMSO-d_6, 75.47 MHz) δ: 174.1 (C-9), 169.3 (NH*C*OOCH_3), 163.3 (C-3/6), 157.4 (C4a/10a), 141.6 (CH=*C*-triazole), 127.5 (C-1/C-8), 125.5 (*C*H=C-triazole), 115.2 (C-8a/9a), 113.8 (C-2/7), 101.5 (C-4/5), 100.7 (C-1'/1''), 77.1 (C-5'/5''), 74.1 (C-4'/4''), 70.5 (C-3'/3''), 66.5 (N-*C*H_2CH_2-N), 61.9 (O-CH_2-triazole), 61.0 (C-6'/6''), 55.1 (C-2'/C-2''), 49.6 (N-*C*H_2CH_2-O), 23.0 (NHCOO*C*H_3).

3.2.12. Synthesis of 3,6-bis(1-(2-(2-amino-3,4,6-hydroxy-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (21)

Compound **20** (0.155 g, 0.18 mmol) was suspended in an aqueous solution of NaOH 20% (15 mL). The mixture was heated at 100ºC for 4 hours. After cooling, the mixture was purified by dialysis using a Spectra/Por 6 regenerated cellulose MWCO 1000 membrane to give 3,6-bis(1-(2-(2-amino-3,4,6-hydroxy-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)-9*H*-xanthen-9-one (**21**) as a dark green solid (0.051 g, 0.064 mmol, 36% yield). mp 157-159 ˚C (H_2O); IR (KBr) υmax: 3362, 2920, 2880, 1641, 1609, 841; ^{1}H NMR (DMSO-d_6, 300.13 MHz) δ: 8.37 (1H, *s*, *H*-triazole), 8.08 (1H, *d*, *J*=8.9 Hz, H-1/8), 7.33 (1H, *brd*, H-4/5), 7.11 (1H, *d*, *J*= 8.8 Hz, H-2/7), 5.35 (2H, *s*, OCH_2-triazole), 5.04 (1H, *brd*, OH), 4.96 (1H, *s*, OH), 4.62-4.55 (3H, *m*, N-CH_2-CH2-O, OH), 4.12-4.10 (2H, *m*, N-CH_2-CH_2, H1'/1''), 3.91-3.87 (1H, *m*, N-CH_2-CH_2), 3.71-3.67 (2H, *m*, H-6'/6''a,b), 3.11-3.04 (4H, *m*, H- 2'/2'',H-3'/3'', H-4'/4'', H-5'/5'') 1.44 (2H, *s*, NH_2); ^{13}C NMR (DMSO-d_6, 75.47

MHz) δ: 174.0 (C-9), 163.3 (C-3/6), 157.5 (C-4a/10a), 141.7 (CH=C-triazole), 127.6 (C-1/8), 125.8 (CH=C-triazole), 115.2 (C-8a/9a), 113.9 (C-2/C-7), 101.5 (C-4/C-5, C-1'/1''), 77.2 (C-5'/C-5''), 76.5 (C-4'/4'') 70.9 (C-3'/3''), 67.3 (N-CH_2*C*H_2-O), 61.9 (O-*C*H_2-triazole), 61.1 (C-6'/C-6''), 49.8 (C-2'/2''), 49.6 (N-*C*H_2CH_2-O).

3.2.13. Synthesis of 3,6-bis(1-(2-(2-amino-3,4,6-tri-*O*-sulfate-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)xanthone (22)

Compound **21** (0.045 g, 0.05 mmol) was dissolved in DMA (5 mL) and the mixture was kept under MW irradiation for 15 minutes. Then, SO_3:TEA adduct (0.046 g, 0.057 mmol, 10 eq./OH and NH_2) was added. The reaction solution was kept MW irradiation (200W) at 100 ºC for 2 hours. After cooling, TEA was added until the pH was 8 and the obtained solution was poured into acetone and left at 4ºC for 24 hours. The crude oil formed was washed with acetone and ether to remove unreacted adduct and then was dissolved in aqueous solution of 30% NaOAc (2 mL). EtOH was added and the sodium salt of 3,6-bis(1-(2-(2-amino-3,4,6-tri-*O*-sulfate-2-deoxy-*β*-D-glucopyranosyl)ethyl)-1*H*-1,2,3-triazole-4-yl)methoxy)-9*H*-xanthen-9-one (**22**) insolubilized as a dark brown solid that was further purified by dialysis using a Spectra/Por 6 regenerated cellulose MWCO 1000 membrane (0.055 g, 0.04 mmol, 78% yield); mp 192 ˚C dec (H_2O); IR (KBr) υmax: 2917, 2847, 1642, 1619, 1257, 1047, 814; ^{1}H NMR (DMSO-d_6, 300.13 MHz) δ: 8.27 (1H, *s*, CH=C-triazole), 8.07 (1H, *d*, *J*=8.8 Hz, H-1/8), 7.35 (1H, *brd*, H4/5), 7.13 (1H, *d*, *J*=7.5 Hz, H-2/7), 5.33 (2H, *s*, OCH_2-triazole), 4.63-4.60 (2H, *m*, N-CH_2CH_2-O), 4.15-4.08 (3H, *m*, H-1'/1'', N-CH_2CH_2-O), 3.84-3.30 (3H, *m*, H-2/2', H-3'/3'', H-4'/4'' and H5'/5'', under water), 1.73 (2H, *s*, N*H*$_2$); ^{13}C NMR (DMSO-d_6, 75.47 MHz) δ: 174.2 (C-9), 163.4 (C3/6), 157.5 (C-4a/10a), 141.7 (CH=*C*-triazole), 127.5 (C-1/8), 125.5 (*C*H=C-triazole), 115.2 (C-8a/9a), 113.9 (C-2/7), 101.5 (C-4/5), 100.5 (C-1'/1''), 76.9 (C-5'/5''), 74.5 (C-4'/4''), 70.5 (C-3'/3''), 66.1 (N-CH_2CH_2-N), 61.9 (O-C*H*$_2$-triazole), 60.9 (C-6'/6''), 53.0 (C-2'/2''), 49.5 (N-CH_2CH_2-N).

3.2.14. Synthesis of diosmin peracetate (24)

Diosmin (**23**, D3525, 0.05 g, 0.08 mmol) and NaF (0.42 g, 10 mmol) were mixed in acetic anhydride (4 mL) and the mixture was kept under MW irradiation (400W) at 130ºC for 35 minutes. After cooling, the solution obtained was poured into ice and then extracted with CH_2Cl_2. The organic layer was extracted with $NaHCO_3$, dried over anhydrous Na_2SO_4 and filtered. Crystallization from MeOH/H_2O furnished 5,3-*O*-acetyl-2-(3-*O*-acetyl-4-methoxyphenyl)-7-[2,3,4-tri-*O*-acetyl-*α*-L-rhamnopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-*β*-D-

glucopyranosyloxy]oxychromen-4-one (**24**) as a yellow solid (0.048 g, 0.051 mmol, 62% yield). mp 135-138 ˚C; IR (KBr) υmax: 2940, 1754, 1644, 1614, 1514, 1433, 1371, 1289, 982 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz) *δ*: 7.72 (1H, *dd*, *J*= 2.3, 9.0 Hz, H-6'), 7.56 (1H, *d*, *J*= 2.3 Hz, H-2'), 7.08 (1H, *d*, *J*= 8.8 Hz, H-5'), 6.96 (1H, *d*, *J*= 2.4, H-8), 6.65 (1H, *d*, *J*= 2.4, H-6), 6.51 (1H, *s*, H-3), 5.37-5.15 (6H, *m*, H-1'', H-2'', H-3'', H-4'', H-3''', H-4'''), 5.05-4.97 (1H, *m*, H-2'''), 4.72 (1H, *s*, H-1'''), 4.01-3.95 (1H, *m*, H-5'''), 3.91 (3H, *s*, 4'-OC*H*$_3$), 3.85-3.80 (2H, *m*, H-5'', 6''a), 3.70-3.65 (1H, *m*, H-6''b), 2.43 (3H, *s*, $COCH_3$), 2.36 (3H, *s*, $COCH_3$), 2.17 (3H, *s*, $COCH_3$), 2.09-2.02 (16H, *m*, $COCH_3$), 1.92 (3H, *s*, $COCH_3$), 1.15 (3H, *d*, *J*=6.2, H-6'''); ^{13}C NMR ($CDCl_3$, 75.47 MHz) *δ*: 176.2 (C-4), 170.2 (*C*OCH_3), 170.0 (*C*OCH_3), 169.9 (*C*OCH_3), 169.8 (*C*OCH_3), 169.7 (*C*OCH_3), 169.4 (*C*OCH_3), 169.3 (*C*OCH_3), 168.8 (*C*OCH_3), 161.5 (C-7), 159.8 (C-2, C-5), 154.0 (C-4'), 150.7 (C-9), 140.1 (C-3'), 123.9 (C-1'), 121.0 (C-6'), 112.8 (C-5'), 112.5 (C-2'), 109.0 (C-3), 102.4 (C-10), 98.1 (C-6, C-1'''), 97.8 (C-8, C-1''), 73.5 (C-5''), 72.4 (C-3''), 70.9 (C-2''), 70.8 (C-4'', C-4'''), 69.3 (C-2'''), 68.9 (C-3'''), 68.6 (C-6''), 66.7 (C-5'''), 56.2 (4'-OCH_3), 31.0 (CO*C*H_3), 29.7 (CO*C*H_3), 21.1 (CO*C*H_3), 20.8 (CO*C*H_3), 20.8 (CO*C*H_3), 20.7 (CO*C*H_3), 20.6 (CO*C*H_3),17.3 (C-6''').

3.2.15. Synthesis of rutin peracetate (26)

Rutin (**25**, R2303, 0.3 g, 0.5 mmol) was mixed with acetic anhydride (12 mL) and the mixture was heated at 130˚C until completion of the reaction confirmed by TLC. The solution obtained was poured into ice and extracted with CH_2Cl_2. The organic layer was extracted with a saturated solution of $NaHCO_3$, dried with anhydrous Na_2SO_4, filtered, and then evaporated under reduced pressure. The obtained oil was dissolved in ethyl acetate and insolubilized with petroleum ether 60-80˚C and 2-(3,4-di-*O*-acetylphenyl)-5,7-di-*O*-acetyl-3-[3,4,5-tri-*O*-acetyl-*α*-L-rhamnopyranosyl-(1→6)-3,4,5-tri-*O*-acetyl-*β*-D-glucopyranosyloxy]-4*H*-chromen-4-one (**26**) was obtained as a white solid (0.43 g, 0.41 mmol, 73% yield). mp 119-120 ˚C (petroleum ether 60-80˚C); IR (KBr) υmax: 2940, 1781, 1754, 1628, 1505, 1477, 1435, 1371, 1145 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz) δ: 7.96 (1H, *dd*, *J*=2.2 and 8.6 Hz, H-6'), 7.91 (1H, *d*, *J*=2.1 Hz, H-2'), 7.34 (1H, *d*, *J*=8.6 Hz, H-5'), 7.31 (1H, *d*, *J*=2.2 Hz, H-8), 6.83 (1H, *d*, *J*=2.2 Hz, H-6), 5.43 (1H, *d*, *J*=7.8 Hz, H-1''), 5.28 (1H, *t*, *J*=9.6 Hz, H-3''), 5.17 (1H, *dd*, *J*=7.8 and 9.8 Hz, H-2''), 5.09-5.06 (2H, *m*, H-2''', H-3'''), 4.95 (2H, *t*, *J*=9.7 Hz, H-4'', 4'''), 4.52 (1H, *brs*, H-1'''), 3.67-3.62 (1H, *m*, H-5'''), 3.60-3.54 (1H, *m*, H-5''), 3.53 (1H, *dd*, *J*=3.1 and 12.5 Hz, H-6a), 3.26 (1H, *dd*, *J*=5.9 and 11.3 Hz, H-6''b), 2.44 (3H, *s*, $COCH_3$), 2.35 (3H, *s*, $OCOCH_3$), 2.34 (3H, *s*, $OCOCH_3$), 2.30 (3H, *s*, CO*C*H_3), 2.14 (3H, *s*, $COCH_3$), 2.09 (3H, *s*, $COCH_3$), 2.03 (6H, *s*, $COCH_3$), 1.96 (3H, *s*, $COCH_3$), 1.94 (3H, *s*, $COCH_3$), 1.60 (3H, *s*, $COCH_3$), 1.06 (3H, *d*, *J*=6.2 Hz, H-6'''); ^{13}C NMR ($CDCl_3$, 75.47 MHz)

δ: 171.9 (C-4), 170.2 (*C*OCH$_3$), 169.9 (*C*OCH$_3$), 169.9 (*C*OCH$_3$), 169.8 (*C*OCH$_3$), 169.8 (*C*OCH$_3$), 169.6 (*C*OCH$_3$), 169.3 (*C*OCH$_3$), 168.1 (*C*OCH$_3$), 167.9 (*C*OCH$_3$), 167.8 (*C*OCH$_3$), 156.6 (C-2), 154.7 (C9), 153.9 (C-7), 150.2 (C-5), 144.1 (C-4'), 141.8 (C-3'), 136.9 (C-3), 128.6 (C-1'), 127.2 (C-6'), 124.7 (C-2'), 123.5 (C-5'), 115.1 (C-10), 113.4 (C-6), 109.1 (C-8), 99.6 (C-1''), 97.7 (C-1'''), 72.8 (C-5''), 72.5 (C-3''), 71.4 (C-2''), 70.9 (C-4'''), 69.5 (C-4''), 69.3 (C-2'''), 69.0 (C-3'''), 66.9 (C-6''), 66.3 (C-5'''), 21.2 (CO*C*H$_3$), 21.1 (CO*C*H$_3$), 20.9 (CO*C*H$_3$), 20.9 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.7 (CO*C*H$_3$), 20.6 (CO*C*H$_3$), 17.2 (C-6''').

3.2.16. Synthesis of naringin persulfate (27)

Naringin (**8**, N1376, 0.5 g, 0.86 mmol) was dissolved in DMA (10 mL) and SO_3:TEA adduct (9.37 g; 51.6 mmol; 6 eq./OH) was added. The mixture was kept under MW irradiation (200W) at 100°C for 1 hour. After cooling, TEA was added until the pH was 8 and the obtained solution was poured into acetone and left at 4°C for 24 hours. The crude oil formed was washed with acetone and ether to remove unreacted adduct and then dissolved in aqueous solution of 30% NaOAc (4 mL). EtOH was added and the sodium salt of 7-[[2-*O*-(3,4,6-tri-*O*-sulfate-6-deoxy-*α*-L-mannopyranosyl)-*β*-D-glucopyranosyl]oxy]-2,3-di-*O*-sulfate-5-*O*-sulfate-2-(4-*O*-sulfatephenyl)-4*H*-1-benzopyran-4-one (**27**) insolubilized as yellow solid that was further purified by dialysis using a Spectra/Por 6 regenerated cellulose MWCO 1000 membrane (0.579 g; 0.41 mmol; 48% yield). mp 218-222 ˚C (H_2O); IR (KBr) υmax: 1637, 1518, 1254, 1052, 810; ^{1}H NMR (DMSO-*d*$_6$, 300.13 MHz) δ: 7.58 (2H, *d*, *J*=8.8 Hz, H-2',6'), 7.17 (2H, *d*, *J*=8.7 Hz, H-3', 5'), 6.20-6.18 (1H, brd, H-6), 6.04-5.95 (1H, m, H-8), 5.31-5.23 (2H, *m*, H-1'', 1'''), 4.71-4.69 (1H, *brd*, H-2), 4.56 (1H, *d*, *J*=3.1 Hz, H-neohesperidoside), 4.48 (1H, *s*, H-neohesperidoside) 4.29-4.21 (2H, *m*, H-neohesperidoside), 4.07-3.94 (3H, *m*, H-neohesperidoside), 3.82-3.71 (3H, *m*, H-neohesperidoside), 3.38-3.13 (2H, *m*, H-3, under water), 1.27 (3H, *d*, *J*=6.1 Hz, 6'''-CH$_3$); ^{13}C NMR (DMSO-*d*$_6$, 75.47 MHz) *δ*: 197.3 (C-4), 161.3 (C-7, C-9), 157.0 (C-5), 155.6 (C-4'), 129.8 (C-2', C-6'), 129.5 (C-1'), 120.1 (C-3', C-5'), 101.7 (C-10), 97.5 (C-6), 96.1 (C-1''), 96.0 (C-8), 95.5 (C-1'''), 95.2 (C-8), 79.0 (C-2), 76.9-75.3 (3 C-neohesperidoside), 75.0 (C-4'''), 74.3-67.6 (4 C-neohesperidoside), 67.0 (C-5'''), 40.0 (C-3, under DMSO), 18.1 (C-6'''). HRMS (ESI$^+$) m/z calcd for $C_{27}H_{24}O_{38}S_8Na_9$ 1418.6785, found 1418.67605.

3.2. BIOLOGICAL ACTIVITY

3.2.1. Anticoagulant activity

Clotting times were performed in Laboratório de Medicina Laboratorial Dr Carlos da Silva Torres, Grupo Unilabs, with the technical support of Vítor Marques. Human blood was collected from 7 healthy donors aged between 22 and 30 years old without history of bleeding or thrombosis and who had not taken any medication known to affect blood coagulation and platelet function for 2 weeks. Venous blood was obtained and transferred to a plastic tube. Nine volumes of blood were decalcified with one volume of 3.8% sodium citrate solution. Blood was centrifuged for 20 min at 2400g and the pooled plasma was stored in aliquots at -20 °C until use. Sulfated compounds were dissolved in saline solution. The final concentration of sulfated compounds in these assays ranged from 1.0×10^{-3} to 2.56×10^{-5} M. Saline was used as control.

Activated partial thromboplastin time (APTT): The assay was carried out according to the respective instructions of the manufacture: citrated normal human plasma mixed (1:1) with sample solution (50 µL), and APTT assay reagent (50 µL) and the mixture was incubated for 6 min at 37°C. $CaCl_2$ (50 µL, 25 mmol/L) was added, and clotting times were recorded during 180 s. The APTT reagent used was the synthetic phospolipid cephaline with extract of rabbit brain tissue, and a polyphenolic activator.

Prothrombin time (PT): The assay was carried out according to the respective instructions of the manufacture: citrated normal human plasma mixed (1:1) with sample solution (50 µL) was incubated. Then PT assay reagent (100 µL) was added and clotting times were recorded during 120 s.

Thrombin time (TT): The assay was carried out according to the respective instructions of the manufacture: citrated normal human plasma mixed (1:1) with sample solution (100 µL) was incubated for 2 min at 37°C. Then 100 µL of thrombin solution (100 U/mL), was added and clotting times were recorded during 240 s. The TT reagent used was 100 U/mL of human calcic thrombin.

The clotting times were recorded in seconds (s). Independent experiences were performed. Coagulation time prolonging ratio was calculated comparing the clotting time in the presence of each concentration of tested compound with that obtained when saline was used instead of test compound. The concentration required to double the clotting time was calculated from linear regression analysis of each individual concentration-response curve.

For clotting time values greater than 240 s (TT), 180 s (APTT), and 120 s (PT), values of 240, 180, and 120 s, respectively, were arbitrarily assigned for statistical analysis.

3.2.2. Statistical analysis

Statistical significance of the difference between control and treated samples was calculated by unpaired *t* test using GraphPad Prism 6 software. A value of $P < 0.05$ was considered significant.

CHAPTER 4 - CONCLUSIONS

Considering the general objectives of this work, the synthesis of new anticoagulant analogues of polysulfated small-molecules with improved oral bioavailability, the main achievements are as following:

- sixteen compounds were obtained by different synthetic methods and ten were characterized for the first time;
- copper(I)-catalyzed alkyne-azide 1,4-cycloaddition and sulfation syntheses were successfully assisted with MW irradiation;
- screening of antitumor activity of some synthetic intermediates revealed a promising hit compound, peracetylated derivative **26**, that deserves further investigation;
- persulfated conjugate of naringin with bile acid **16** and persulfated xanthone triazole-linked glycoside **22** exhibited anticoagulant activity; persulfated naringin-bile acid conjugate **16** was the most potent anticoagulant compound synthesized in LQOF.

The conjugate of naringin with the bile acid DOCA **16** and the xanthone triazole-linked to a glycoside **22** are expected to cross the GI tract membrane: conjugate **16** will be recognized by bile acid transporters and the triazole in compound **22** is expected to increase lipophilicity.

Taking in account the results obtained in this dissertation, the study of the permeability of the synthesized sulfated compounds would be important to perform.

Improving permeability of polysulfated small-molecules could be a step forward to the development of orally active heparin-like antithrombotic drugs.

CHAPTER 5 - REFERENCES

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CHAPTER 6 – APPENDICES

APPENDIX I - ^{1}H and ^{13}C NMR data for compounds **5** (DMSO-d_6), **6** ($CDCl_3$), and **7** ($CDCl_3$).

The NMR data of compound **5** is in accordance with the literature.[75] Values of chemical shift (δ) in parts *per* million (ppm). *J* values (Hz) are presented in parentheses.

	5		**6**		**7**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
1	-	161.8	-	152.9	-	161.3
2	-	107.7	-	118.3	-	118.4
3	-	163.9	-	154.4	-	153.9
4	6.38 (*s*)	93.3	7.25 (*s*)	111.7	6.76 (*s*)	110.6
4a		156.2	-	157.4	-	156.7
5	6.87 (*s*)	102.7	7.39 (*s*)	112.7	7.42 (*s*)	112.9
6	-	154.1	-	152.9	-	148.4
7	-	143.8	-	139.4	-	139.4
8	7.38 (*s*)	108.1	8.00 (*s*)	120.8	8.06 (*s*)	120.1
8a	-	111.7	-	120.1	-	119.9
9	-	179.1	-	174.4	-	180.4
9a	-	150.8	-	112.1	-	106.0
10a	-	101.3	-	147.7	-	148.4
1'	4.59 (*d*, *J*=9.7)	73.1	4.86 (*dd*, *J*=10.1, 4.9)	72.5	5.23-5.17 (*m*)	73.3
2'	4.06 (*t*, *J*=9.2)	70.2	5.73 (*t*, *J*=9.7)	69.4	5.68 (*brs*)	68.3
3'	3.26-3.09 (*m*)	79.0	5.35-5.14 (*m*)	74.4	5.35 (*t*, *J*=9.4)	74.5
4'	3.26-3.09 (*m*)	70.7	5.35-5.14 (*m*)	68.1	5.23-5.17 (*m*)	70.4
5'	3.26-3.09 (*m*)	81.7	3.84-3.80 (*m*)	76.6	3.85-3.81 (*m*)	76.5
6'a	3.69 (*d*, *J*=11.1)	61.5	4.05-3.98 (*d*, *J*=12.6)	61.9	4.04-4.00 (*m*)	62.1
6'b	3.26-3.09 (*m*)		4.42 (*dd*, *J*=12.6, 4.2)		4.40 (*d*, *J*=11.6)	
-OCOCH$_3$	-	-	2.53 (*s*) 2.49 (*s*) 2.34 (*s*) 2.32 (*s*) 2.07 (*s*) 2.06 (*s*) 2.03 (*s*) 1.80 (*s*)	170.6, 170.5 170.3,169.6 168.5, 168.0 167.7, 167.2 21.4, 21.3 21.1, 20.8 20.7, 20.5 20.4, 20.3	2.44 (*s*) 2.35 (*s*) 2.34 (*s*) 2.07 (*s*) 2.05 (*s*) 2.02 (*s*) 1.78 (*s*)	170.5, 170.2 169.6, 169.4 169.2, 168.0 167.1, 22.7, 21.3 20.7, 20.7 20.6, 20.5 20.4
1-OH	13.77 (*s*)	-	-	-	13.25 (*s*)	-
3-OH **6-OH** **7-OH**	10.57 (*brs*)	-	-	-	-	-
2'-OH	4.54 (*s*)	-	-	-	-	-
3',4'-OH,	4.89 (*s*)	-	-	-	-	-
6'-OH	4.52 (*s*)	-	-	-	-	-

APPENDIX II - ^{1}H and ^{13}C NMR data for compounds **8**, **27**, **11**, and **12** (DMSO-d_6).

Compound **8** was elucidated in accordance with the literature. Values of chemical shift (δ) in parts *per* million (ppm). *J* values (Hz) are presented in parentheses.

	8		**27**		**11**		**12**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2	5.55-5.46 (*m*)	78.9	4.71-4.69 (*m*)	79.0	5.60-5.52 (*m*)	79.2	5.61-5.53 (*m*)	79.2
3	2.78-2.67 (2H, *m*)	42.0	3.38-3.13 (2H, *m*)#	-*	2.82-2.73 (2H, *m*)	45.4	2.82-2.71 (2H, *m*)	42.0
4	-	197.3	-	197.3	-	197.2	-	197.2
5	12.05 (OH, *s*)	162.9	-	157.0	12.05 (OH, *s*)	163.0	12.05 (OH, *s*)	163.3
6	6.09-6.08 (*m*)	96.3	6.20-6.18 (*brd*)	97.1	6.13-6.08 (*m*)	96.4	6.16-6.08 (*m*)	96.3
7	-	164.8	-	161.3	-	164.8	-	164.8
8	6.11 (*d*, *J*=2.0)	95.2	6.04-5.95 (*m*)	96.0	6.20 (*d*, *J*=2.5)	95.2	6.16-6.08 (*m*)	95.2
9	-	162.8	-	161.3	-	162.8	-	162.8
10	-	103.3	-	103.3	-	103.4	-	103.3
1'	-	128.7	-	129.5	-	128.5	-	128.5
2'	7.33 (*dd*, *J*=8.7, 2.4,)	128.6	7.58 (*d*, *J*=8.8)	129.8	7.46 (*dd*, *J*=8.9, 2.4)	128.4	7.44 (*dd*, *J*=8.8, 2.4)	128.4
3'	6.80 (*d*, *J*=8.5)	115.3	7.17 (*d*, *J*=8.7)	120.1	7.02-6.97 (*m*)	115.1	6.93 (*d*, *J*=8.8)	115.0
4'	9.65 (OH, *s*)	157.9	-	155.6	-	157.9	-	157.9
5'	6.80 (*d*, *J*=8.5)	115.3	7.17 (*d*, *J*=8.7)	120.1	7.02-6.97 (*m*)	115.1	6.93 (*d*, *J*=8.8)	115.0
6'	7.33 (*dd*, *J*=8.7, 2.4)	128.5	7.58 (*d*, *J*=8.8)	129.8	7.46 (*dd*, *J*=8.9, 2.4)	128.2	7.44 (*dd*, *J*=8.8, 2.4)	128.3
1''	5.14-5.09 (*m*)	100.5	5.31-5.23 (*m*)	96.1	5.15-5.10 (*m*)	100.5	5.17-5.05 (*m*)	100.5
2''	3.48-3.29 (*m*)#	77.1	4.71-3.56 (*m*)	66.9-76.9	3.54-3.30 (*m*)#	77.1	3.49-3.16 (*m*)#	77.1
3''		76.1				76.1		76.1
4''	3.23-3.15 (*m*)	69.6			3.23-3.15 (*m*)	69.6	3.25-3.16 (*m*)	69.6
5''	3.48-3.29 (*m*)#	76.9			3.54-3.30 (*m*)#	76.9	3.49-3.16 (*m*)#	76.9
6''a	3.70-3.65 (*m*)	60.5			3.74-3.64 (*m*)	60.5	3.74-3.64 (*m*)	60.5
6''b	3.48-3.29 (*m*)#				3.54-3.30 (*m*)#		3.49-3.16 (*m*)#	
1'''	5.14-5.09 (*m*)	97.3	5.31-5.23 (*m*)	96.1	5.15-5.10 (*m*)	97.3	5.17-5.05 (*m*)	97.4
2'''	3.70-3.65 (*m*)	70.5	4.71-3.56 (*m*)	66.9-76.9	3.74-3.64 (*m*)	70.5	3.25-3.16 (*m*)	70.5
3'''	3.23-3.15 (*m*)	70.4			3.23-3.15 (*m*)	70.4		70.4
4'''		71.8		72.1		71.8		71.8
5'''	3.70-3.65 (*m*)	68.3		67.0	3.74-3.64 (*m*)	68.3	3.74-3.64 (*m*)	68.3
OH	5.33 (d, *J*=4.8)	-	-	-	5.15-5.10 (*m*)	-	5.17-5.05 (*m*)	-
OH	5.15-5.09 (*m*)	-	-	-		-		-
OH	4.60-4.58 (*m*)	-	-	-	4.92-4.83 (*m*)	-	4.51 (*m*)	-
OH	4.68 (*d*, *J*=4.3)	-	-	-		-	4.57 (*d*, *J*=4.4)	-
OH	4.50 (*d*, *J*=5.7)	-	-	-		-	4.43-4.38 (*m*)	-
OH	4.74 (*d*, J=4.6)	-	-	-		-	4.62 (*d*, *J*=3.5)	-
6'''-CH_3	1.15 (*d*, *J*=6.1)	18.1	1.27 (*d*, *J*=6.1)	18.1	1.15 (*d*, *J*=6.2)	18.1	1.16 (*d*, *J*=6.2)	18.1
O-CH_3	-	-	-	-	3.71 (*s*)	51.9	-	-
-$COCH_3$	-	-	-	-	-	169.2	-	-
O-CH_2-	-	-	-	-	4.83 (*s*)	65.4	4.62 (*s*)	65.3
-COOH	-	-	-	-	-	-	13.89 (*s*)	170.1

*****Under DMSO signal; #under water signal.

APPENDIX III - ^{1}H and ^{13}C NMR data for compounds **9** (DMSO-d_6), **13** ($CDCl_3$), and **14** (DMSO-d_6).

Compound **9** was elucidated in accordance with the literature.[139] Values of chemical shift (δ) in parts *per* million (ppm). *J* values (Hz) are presented in parentheses.

	9		**13**		**14**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
1a,b	1.81-0.95 (*m*)	35.0	1.94-1.05 (*m*)	34.9	1.82-0.97 (*m*)	35.1
2a,b		30.3		30.5	1.82-0.97 (*m*)	28.7
3	3.79 (*d*, *J*=2.4)	70.0	3.98-3.56 (*m*)	71.8	3.79 (*s*)	70.0
3-OH	4.48 (*d*, *J*=2.6)	-	4.00 (*t*, *J*=2.8)	-	4.49 (*s*)	-
4	1.81-0.95 (*m*)	35.7	1.94-1.05 (*m*)	36.0	1.82-0.97 (*m*)	35.7
5		41.6		42.1		42.3
6		27.2		27.4		27.2
7		26.1		26.1		26.1
8		36.3		36.4		36.3
9		32.9		33.7		32.9
10		33.8		34.1		33.8
11		28.6		28.0		28.6
12	4.21 (*d*, *J*=3.9)	71.0	3.77-3.73 (*m*)	73.1	4.21 (*brd*)	71.0
13	1.81-0.95 (*m*)	46.0	1.94-1.05 (*m*)	46.6	1.82-0.97 (*m*)	46.0
14	-	47.5	-	48.2	-	47.5
15	-	23.5	-	23.6	-	23.5
16	-	27.0	-	27.1	-	27.0
17	-	46.2	-	47.1	-	46.2
18-CH_3	0.59 (*s*)	12.5	0.70 (*s*)	12.7	0.59 (*s*)	12.5
19-CH_3	0.84 (*s*)	23.1	0.91 (*s*)	23.2	0.84 (*s*)	23.1
20	1.81-0.95 (*m*)	35.2	1.94-1.05 (m)	35.2	1.82-0.97 (*m*)	35.1
21-CH_3	0.91 (*d*, *J*=6.2)	16.9	1.01 (*d*, *J*=6.1)	17.3	0.92 (*d*, *J*=6.2)	17.1
22	1.81-0.95 (*m*)	30.9	1.94-1.05 (*m*)	30.5	1.82-0.97 (*m*)	32.9
23a		30.8	2.67-2.55 (*m*)	30.5	2.13-2.04 (*m*)	32.5
23b	2.12-2.09 (*m*)				2.00-1.90 (*m*)	
24	-	175.0	-	169.1	-	172.7
-CH_2-CH_2-(NHS)	-	-	2.87-2.82 (*m*)	25.6	-	-
C=O (NHS)	-	-	-	169.2	-	-
-NH-	-	-	-	-	7.72 (*t*, *J*=10.9)	-
-NH_2	-	-	-	-	1.82-0.97 (*m*)	-
CH_2-CH_2 (EDA)	-	-	-	-	3.01 (*q*, *J*=12.2, 6.3)	41.6 41.4

APPENDIX IV - ^{1}H and ^{13}C NMR data for compounds **10**, **15,** and **16** (DMSO-d_6).

Values of chemical shift (δ) in parts *per* million (ppm). *J* values (Hz) are presented in parentheses.

	10		**15**		**16**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
2	5.70-5.61 (*m*)	77.0	5.54-5.50 (*m*)	77.4	5.35-5.20 (*m*)	78.8
3	2.89-2.87 (*m*) 2.83-2.81 (*m*)	41.6	2.89-2.71 (*m*)	46.4	2.72-2.61 (*m*)	45.6
5	12.03 (OH, *s*)	163.0	12.06 (OH, *s*)	163.0	-	163.4
6,8	6.15-6.09 (*m*)	97.0 95.0	6.21-6.10 (*m*)	96.8	6.19-5.98 (*m*)	96.0
7	-	162.8	-	164.8	-	164.7
2',6'	7.56 (*d*, *J*=8.1)	128.0	7.87-7.79 (*m*) 7.49-7.45 (*brd*)	128.9	7.90 (*brd*) 7.49 (*brd*)	128.0
3',5'	7.17 (*d*, *J*=8.5)	122.0	7.03-6.99 (*brd*)	115.6	7.03 (*m*)	114.8
1''	5.09 (*brd*)	100.7	5.18-5.10 (*m*)	100.9	5.35-5.20 (*m*)	100.8
1'''	5.20-5.16 (*m*)	97.0		97.4		97.3
neohesperidoside	3.73-3.67 (*m*) 3.51-3.28 (*m*) 3.23-3.16 (*m*)	71.1-68.0	3.77-3.69 (*m*) 3.55-3.35 (*m*)*	76.6-60.9	4.75-4.68 (*m*) 4.53-4.42 (*m*) 4.01-3.70 (*m*)	76.1-71.5
6'''-CH_3	1.17 (*d*, *J*=6.2)	18.1	1.75-1.14 (*m*)	18.4	1.90-1.16 (*m*)	18.0
OH	5.42 (*d*, *J*=5.2) 5.47 (*d*, *J*=4.6) 4.75 (*dd*, *J*=4.2, 2.3) 4.69 (*d*, *J*=4.3)	-	4.77-4.53 (*m*) 5.36-5.32 (*m*)	-	-	-
-OCH_2-	-	-	4.77-4.53 (*m*)	67.2	4.53-4.42 (*m*)	67.0
3 (D)	3.81 (*d*, *J*=2.9, OH) 3.73-3.67 (m)	70.0	4.77-4.53 (OH, *m*) 3.77-3.69 (*m*)	70.5	4.01-3.70 (*m*)	76.1
12 (D)	4.26 (*d*, *J*=2.9)	71.1	4.19 (*brd*)	71.6	4.53-4.42 (*m*)	78.0
steroid	1.82-1.16 (*m*)	47.5-23.5	1.75-1.14 (*m*)	47.8-23.9	1.90-1.16 (*m*)	48.2-23.6
18-CH_3	0.63 (*s*)	12.4 12.5	0.59 (*s*)	12.8	0.62 (*s*)	12.3
19-CH_3	0.85 (*s*)	23.1	0.84 (*s*)	23.5	0.85 (*s*)	23.2
21-CH_3	0.99 (*d*, *J*=5.7)	16.7 17.0	0.91 (*d*, *J*=6.0)	17.4	0.94 (*d*, *J*=6.4)	17.4
23 (D)	2.28-2.18 (*m*)	30.6	2.07-1.91 (*m*)	31.6	2.13-2.09 (*m*)	31.6
N-CH_2CH_2-N	-	-	3.18-3.15 (*m*)	38.5	3.16-3.15 (*m*)	38.2 38.4
NH-amide	-	-	8.17 (*s*) 7.87-7.79 (*m*)	174.3 168.6	8.24 (*s*) 7.90 (*brd*)	173.1 167.8

*under water signal

APPENDIX V - ^{1}H and ^{13}C NMR data for compounds **19**, **20**, **21**, and **22** (DMSO-d_6).

For all the compounds sugar protons were assigned using the ^{1}H and ^{13}C data of glucosamine. [140] Values of chemical shift (δ) in part *per* million (ppm). *J* values (Hz) are presented in parentheses.

	19		**20**		**21**		**22**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1/8	8.09 (*d*, *J*=8.9)	127.5	8.10 (*d*, *J*=9.0)	127.5	8.08 (*d*, *J*=8.9)	127.6	8.07 (*d, J*=8.8)	127.5
2/7	7.10 (*dd*, *J*=9.0, 2.3)	113.8	7.12 (*dd*, *J*=8.9, 2.1)	113.8	7.11 (*d*, *J*=8.9)	113.9	7.13 (*d*, *J*=7.5)	113.9
3/6	-	163.3	-	163.3	-	163.3	-	163.4
4/5	7.34 (*d*, *J*=2.3)	101.5	7.35 (*d*, *J*=2.1)	101.5	7.33 (*brd*)	101.5	7.35 (*brd*)	101.5
4a/10a	-	157.4	-	157.4	-	157.5	-	157.5
9	-	174.1	-	174.1	-	174.0	-	174.2
8a/9a	-	115.0	-	115.2	-	115.2	-	115.2
1'/1''	4.70 (d, *J*=8.5)	99.8	4.33 (*d*, *J*=8.4)	100.7	4.61-4.55 (*m*)	101.5	4.15-4.08 (*m*)	100.5
2'/2''	3.76 (*q*, *J*=8.9)	52.8	3.49-3.40 (*m*)*	55.1	3.11-3.04 (*m*)	49.8	3.84-3.30 (*m*)*	53.0
3'/3''	4.85 (*t*, *J*=9.7)	68.4	3.18-3.04 (*m*)	70.5		70.9		70.5
4'/4''	4.21-4.02 *(m)*	70.7	3.29-3.25 *(m)*	74.1		76.5		74.5
5'/5''	5.07 (*t*, *J*= 10.0)	72.5	3.18-3.04 *(m)*	77.1		77.2		76.9
6'/6''a,b	4.21-4.02 (*m*)	61.7	3.73-3.68 *(m)*	61.0	3.71-3.67 (*m*)	61.1		61.9
-OH	-	-	5.08 (*brd*)	-	5.04 (*brd*)	-	-	-
-OH	-	-	5.01 (*brd*)	-	4.96 (*brd*)	-	-	-
-OH	-	-	4.60-4.57 (*m*)	-	4.62-4.55 (*m*)	-	-	-
O-CH_2-triazole	5.33 *(s)*	61.9	5.33 *(s)*	61.9	5.35 (*s*)	61.9	5.33 (*s*)	61.9
CH=C-triazole	8.15 *(s)*	125.4 141.5	8.18 *(s)*	125.5 141.6	8.37 (*s*)	125.8 141.7	8.27 (*s*)	125.5 141.7
N-CH_2CH_2-O	4.60-4.57 (*m*) 4.21-4.02 (*m*)	67.2 49.4	4.60-4.57 *(m)* 4.12-4.08 (*m*) 3.87-3.81 (*m*)	66.5 49.6	4.62-4.55 (*m*)	67.3 49.8	4.15-4.08 (*m*) 4.63-4.60 (*m*)	66.1 49.5
-$OCOCH_3$	2.02 (*s*) 1.97 (*s*) 1.91 (*s*)	170.1 169.7 169.4 20.5 20.4 20.3	-	-	-	-	-	-
-NH*COCH*$_3$	1.71 (*s*)	169.1 22.6		169.3 23.0	-	-	-	-
-N*H*$COCH_3$	7.92 (*d*, *J*=9.1)	-	7.70 (*d*, *J*=8.9)	-	-	-	-	-
-NH_2	-	-	-	-	1.44 (*s*)	-	1.73 (*s*)	-

*under water

APPENDIX VI - ^{1}H and ^{13}C NMR data for compounds **23** (DMSO-d_6) and **24** ($CDCl_3$).

Data for compound **23** is in accordance with the literature.[141] Values of chemical shift (δ) in parts *per* million (ppm). *J* values (Hz) are presented in parentheses.

	23		**24**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**
2	-	164.5		159.8
3	6.79 (*s*)	105.7	6.51 (*s*)	109.0
4	-	182.3		176.2
5	12.95 (*s*, OH)	161.1	-	159.8
6	6.44 (*d*, *J*=1.8)	99.9	6.65 (*d*, *J*=2.4)	98.1
7	-	163.2	-	161.5
8	6.75 (*d*, *J*=1.8)	95.1	6.96 (*d*, *J*=2.4)	97.8
9	-	157.2	-	150.7
10	-	104.0	-	102.4
1'	-	123.0	-	123.9
2'	7.41 (*d*, *J*=2.0)	112.9	7.56 (*d*, *J*=2.3)	112.5
3'	9.73 (*brs*, OH)	146.9	-	140.1
4'	-	151.6	-	154.0
4'-OCH_3	3.84 (*s*)	56.0	3.91 (*s*)	56.2
5'	7.10 (*d*, *J*=9.0)	113.3	7.08 (*d*, *J*=8.8)	112.8
6'	7.54 (*dd*, *J*=9.0, 2.0)	119.3	7.72 (*dd*, *J*=9.0, 2.3)	121.0
1''	4.51 (*d*, *J*=7.0)	100.1	5.98-5.37 (*m*)	97.8
2''	3.75-3.13 (*m*)	73.3		70.9
3''		76.4		72.4
4''		70.9		70.8
5''		75.8	3.85-3.80 (*m*)	73.5
6''a, b		66.2	3.85-3.80 (*m*) 3.70-3.65 (*m*)	68.6
1'''	5.03 (*d*, *J*=7.0)	100.7	4.72 (*s*)	98.1
2'''	3.75-3.13 (*m*)	70.5	5.05-4.97 (*m*)	69.3
3'''		69.8	5.98-5.37 (*m*)	68.9
4'''		72.3		70.8
5'''		68.6	4.01-3.95 (*m*)	66.7
6'''-CH_3	1.04 (*d*, *J*=6.1)	18.0	1.15 (*d*, *J*=6.2)	17.3
OH	5.55 (*d*, *J*=4.4)	-	-	-
OH	5.30 (*d*, *J*=5.4)	-	-	-
OH	5.25 (*d*, *J*=4.3)	-	-	-
OH	4.80 (*m*)	-	-	-
OH	4.69 (*m*)	-	-	-
OH	4.52 (*m*)	-	-	-
-$COCH_3$	-	-	2.43 (*s*) 2.36 (*s*) 2.17 (*s*) 2.09-2.02 (*m*) 1.92 (*s*)	170.2, 170.0 169.9, 169.8 169.7, 169.4 169.3, 168.8 31.0, 29.7 21.1, 20.8 20.8, 20.7, 20.6

APPENDIX VII - ^{1}H and ^{13}C NMR data for compounds **25** (DMSO-d_6) and **26** ($CDCl_3$).

Compound **25** was elucidated in accordance with the literature.[74, 129] Values of chemical shift (δ) in parts *per* million (ppm). *J* values (Hz) are presented in parentheses.

	25		**26**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**
2	-	156.5	-	156.6
3	-	133.3	-	136.9
4	-	177.4	-	171.9
5	12.60 (*s*, OH)	161.3	-	150.2
6	6.21 (*d*, *J*=1.9)	98.8	6.83 (*d*, *J*=2.2)	113.4
7	10.77 (*s*, OH)	164.1	-	153.9
8	6.40 (*d*, *J*=1.9)	100.8	7.31 (*d*, *J*=2.2)	109.1
9	-	156.7	-	154.7
10	-	101.2	-	115.1
1'	-	121.2	-	128.6
2'	7.54 (*m*)	115.3	7.91 (*d*, *J*=2.1)	124.7
3'	9.60 (OH, *s*)	144.8	-	141.8
4'	9.17 (OH, *s*)	149.5	-	144.1
5'	6.85 (*d*, *J*=9.0)	116.3	7.34 (*d*, *J*=8.6)	123.5
6'	7.56 (*m*)	121.7	7.96 (*dd*, *J*=8.6, 2.2)	127.2
1''	5.37-5.34 (*m*)	93.7	5.43 (*d*, *J*=7.8)	99.6
2''	3.34-3.23 (*m*)	76.5	5.17 (*dd*, *J*=9.8, 7.8)	71.4
OH	5.27 (*m*)	-	-	-
3''	3.34-3.23 (*m*)	144.8	5.28 (*t*, *J*=9.6)	72.5
OH	5.07 (*m*)	-	-	-
4''	3.12-3.06 (*m*)	148.5	4.95 (*t*, *J*=9.7)	69.5
OH	5.07 (*m*)	-	-	-
5''	3.34-3.23 (*m*)	71.9	3.60-3.54 (*m*)	72.8
6''a	3.72 (*d*, *J*=10.1)	67.1	3.53 (*dd*, *J*=12.5, 3.1)	66.9
6''b	3.34-3.23 (*m*)		3.26 (*dd*, *J*=11.3, 5.9)	
1'''	4.40 (*m*)	104.0	4.52 (*brs*)	97.7
2'''	3.34-3.23 (*m*)	70.6	5.09-5.06 (*m*)	69.3
3'''		70.4		69.0
4'''	3.12-3.06 (*m*)	68.3	4.95 (*t*, *J*=9.7)	70.9
5'''	3.34-3.23 (*m*)	67.1	3.67-3.62 (*m*)	66.3
6'''-CH_3	1.00 (*d*, *J*=6.1)	17.8	1.06 (*d*, *J*=6.2)	17.2
$COCH_3$	-	-	2.44 (*s*), 2.35 (*s*) 2.34 (*s*), 2.30 (*s*) 2.14 (*s*), 2.09 (*s*) 2.03 (*s*), 1.96 (*s*) 1.94 (*s*), 1.60 (*s*)	170.2. 169.9 169.9, 169.8 169.8, 169.6 169.3, 168.1 167.9, 167.8 21.2, 21.1 20.9, 20.9 20.7, 20.7 20.7, 20.6